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VOLUME III, NO. 5 WHOLE NO. 17

SEPTEMBER, 1927

THE AMERICAN JOURNAL OF PATHOLOGY

Official Publication of
The American Association of Pathologists and Bacteriologists

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Editorial and Publication Office
818 HARRISON AVENUE, BOSTON, MASSACHUSETTS
Issued Bimonthly — Annual Subscription in U. S. A. $5.00

Entered as second class matter, March 20, 1914, at the Post Office at Boston, Massachusetts, under the Act of March 3, 1879

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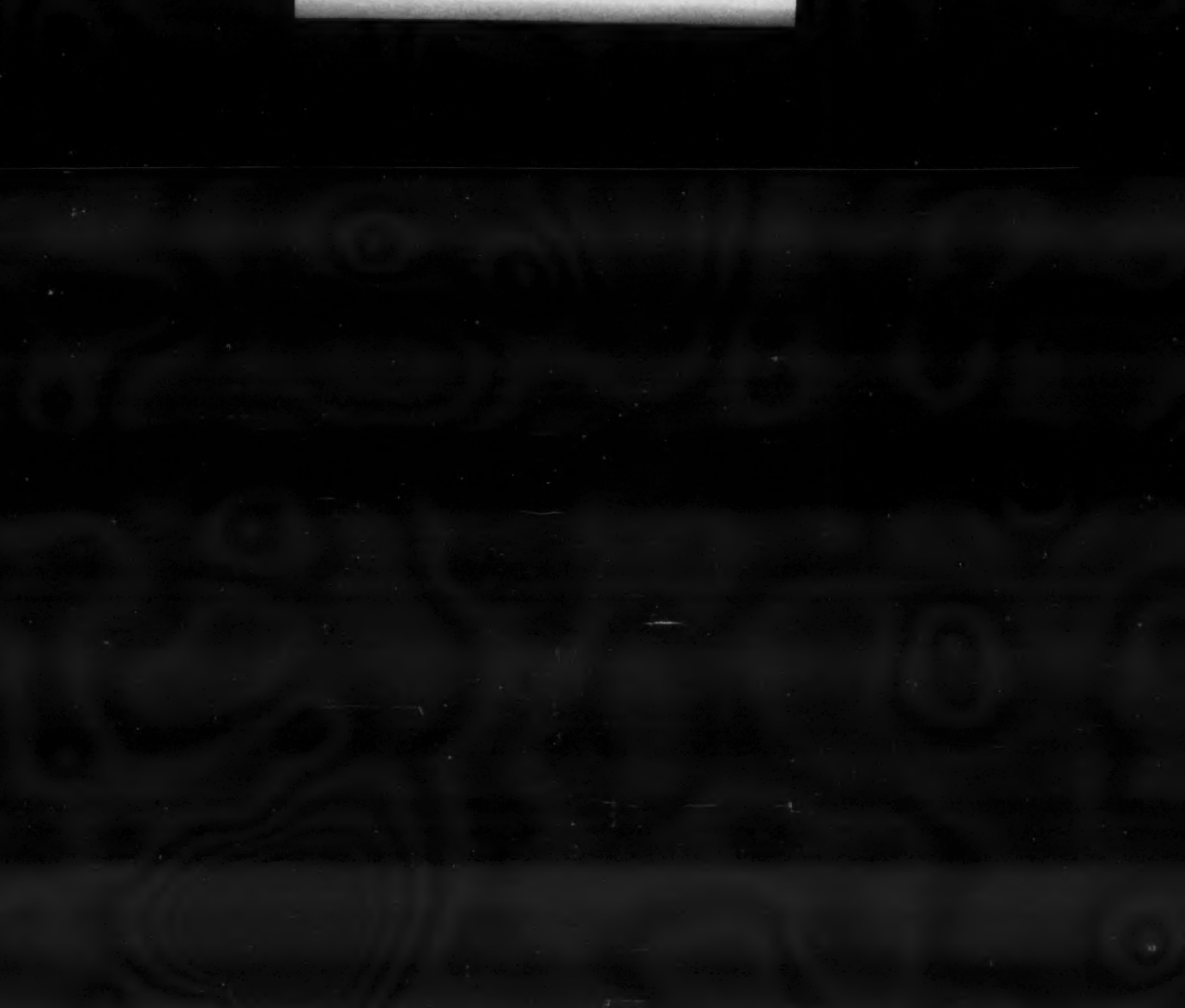
PRINTED AT THE HARVARD UNIVERSITY PRESS, CAMBRIDGE, MASS., U. S. A.



CORRECTION

In Vol. III, No. 3, page 282, first line of second paragraph, for "There is an identity of the individuality differentials," read "An identity of the individuality differentials, that is, a complete loss of the individuality, has not yet been reached within the inbred families."

THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME III SEPTEMBER, 1927 NUMBER 5

STUDIES ON ENDOTHELIAL REACTIONS

X. ON THE ORIGIN OF THE PULMONARY "DUST CELL" *

NATHAN CHANDLER FOOT, M.D.

(From the Department of Pathology, University of Cincinnati College of Medicine and Cincinnati General Hospital, Cincinnati, O.)

INTRODUCTION

It would be very inadvisable to attempt to present the results of the experimental work to be reported in this paper without first reviewing the subject as a whole. So much controversy has centered about the mononuclear phagocytes in general and the pulmonary dust cell in particular that the reader would probably be bewildered by a discussion concerning the latter, were he not permitted to familiarize himself with the views of the various disputants. It will, therefore, be the purpose of this article to afford him this opportunity by presenting a full review of the present status of the doctrine of the dust cell; after which, he may proceed to an enlightened consideration of the value of the data that constitute the original portion of the paper.

Seven years ago, Permar ('20) and Foot ('20), experimenting independently and using somewhat different methods, came to the conclusion that the pulmonary dust cell was derived from the capillary endothelium of the lung. We believed that the capillary endothelium became swollen, its cells proliferated and then migrated into the alveoli, there to become free phagocytes and to multiply still further by mitotic division. We suggested that the phagocytes already present in the alveoli under normal conditions (not called out by a special crisis, such as the injection of dyes or bacteria) had

* Received for publication June 22, 1927.

the same origin, presumably migrating from vascular endothelium from time to time in order to form a sort of scavenging reserve force. We both described cells, which Lang ('25) has subsequently named "septum cells," that are normally present in niches or recesses in the alveolar wall and considered that they had the same origin.

Our theories have been critically studied. They have been accepted either entirely or partially by some and rejected by others. Many investigators agree as to the mesenchymal origin of these cells, although questioning their being derived from the capillary endothelium. A number have put forth well substantiated claims that they arise in the circulating blood as monocytes, migrating to the alveoli as occasion arises. M. R. Lewis ('25) and her pupil Eliot ('25) have employed vital staining to show that the phagocytes are at least of mesenchymal origin, whereas Wislocki ('24) agrees, but remains in doubt as to their precise derivation.

Thus we might be drifting back toward the monocytic origin of these cells, as formerly postulated by Metschnikoff ('07), were our regression not impeded by numerous papers, chiefly by German investigators, who claim that they are merely somewhat altered, desquamated alveolar epithelium. Metschnikoff said: "For long, the large 'dust cells' of the respiratory channels were looked upon as being epithelial cells which were capable of taking up carbon particles, microörganisms and other foreign bodies. In reality these elements are nothing more than white corpuscles that have immigrated into the alveoli and bronchi." The most recent article to support the epithelial side of the argument is by Gross ('27) from Aschoff's laboratory. It will be well to quote at some length from his paper, for in this way one may present the subject in an unbiased fashion, as the statement emanates from the opposing camp and will, for that reason, stress their views rather than ours.

Résumé by Gross: "The ancient dispute concerning the origin of the epithelioid cells has not come to rest since the time of Baumgarten ('01) (fixed tissue cells) and Metschnikoff ('88) (leukocytes). Herxheimer ('03) has injected into the debate, as a third possibility, the participation of the alveolar epithelium. The Japanese school (Kiyono, '14) traces them to histiocytes. Töppich ('25), whose experiments were performed to evaluate Herxheimer's work, champions the endothelial derivation. He sees, as early as one hour after infection, a migration of swollen capillary endothelial cells through

the reticulum of elastic fibrils into the alveolar lumen and their transformation into large mononuclears and epithelioid transitional cells. 'There can be no question of alveolar epithelium, so far as the great majority of the cells is concerned, for disregarding transition-pictures, exactly the same cell types may be recognized as lying definitely within the capillaries.' He explains the circumstance that he found no alveolar epithelium at all by the fact that these cells, 'probably under the direct action' of the large doses of bacilli, had been totally destroyed. Kageyama ('25) found bovine and avian tubercle bacilli, injected in massive doses into the peritoneal cavity, appearing in the pulmonary blood as soon as two hours thereafter. The excretion of these bacilli into the alveoli followed very promptly. After eighteen hours, changes were first noted in the lung tissue, consisting of a proliferation of the alveolar epithelial cells which phagocytosed the bacilli and desquamated into the alveolar lumen as in desquamative catarrh. He never found bacilli in the endothelial cells.

"Tissue cultures have also been drawn upon to solve the problem. Timofejewsky and Benevolenskaja ('25) discovered that tubercle bacilli inhibit the growth of rabbit lung tissue cultures. Despite the fact that they established that the particularly active elements in the cultures were the epithelial cells, they do not venture to exclude monocytes, endothelial cells and fibroblasts from the category of bacillary phagocytes, but believe that the epithelioid cells of an organism develop rather from the connective tissue than from the alveolar epithelium. Lang ('25), who denies the existence of alveolar epithelium, finds exudative and productive processes in similar experiments. Pagel ('25) has attempted to answer the question as to the origin of the exudate cells in caseous pneumonia on morphologic grounds. 'In the guinea pig, unmistakable transition pictures as well as structural identity, permit us to trace the exudate cells to the alveolar lining.' Proof in the case of the human subject has fallen flat. ('Ist gescheitert.')."

Further on Gross states: "In the case of the rabbit, I must hold fast to the theory of a primary epithelial reaction." His reason for this is based on the flimsiest of morphologic evidence obtained from the examination of experimental animals a month to six weeks after the injection of tubercle bacilli or dust into the trachea. His argument might be epitomized in the statement: "The dust cells look like epithelium, therefore they are epithelium."

In his summary there is one misstatement that should be corrected; Lang does not deny the existence of alveolar epithelium. He says: "The epithelium is of no importance either, as the alveolar wall, according to my previous investigations, is not provided with any special 'respiratory' epithelial layer, *except the non-nucleated plates.*" The italics are mine. Kageyama's work is not entirely relevant to the discussion, as tuberculosis in the lungs of rats and mice presents an altogether different picture from that which is observed in the case of other mammals.

Here, then, is a statement from the "epithelial camp," whose adherents antedated Herxheimer's "injection" of the epithelial origin of the dust cell into the debate by a number of years, as is indicated by Herxheimer himself. This older literature will be found listed in his paper, in Permar's and in mine ('20).

The Experiments of Westhues and Westhues: Gross merely touches upon the work of the Westhueses ('22, '25), who also adhere firmly to the epithelial origin of the dust cell and have done careful and excellent experiments in an endeavor to prove their case. It has been considered in a former paper (Foot, '25), but it should be mentioned here. In view of the fact that Wislocki ('24) has stated that "Chinesische Tusche" is a colloidal and not a mechanical suspension of carbon as I had thought, it would be well to withdraw my criticism of the work of H. Westhues, wherein I stressed the point that particulate carbon would not mark capillary endothelium.

Westhues noted that there was a decided predilection on the part of the alveolar phacogytes for carbon, carmine being taken up sparingly and slowly. From this he concludes: "As phagocytosis in the lungs takes place more rapidly and energetically than histiocytes could phagocytose, it follows that the engorged (vollgefressenen) cells in the alveoli are not emigrated histiocytes, but could only be alveolar epithelial cells." But, has it been proved that the alveolar epithelium is so energetically phagocytic? Have not the Kupffer cells and splenic phagocytes marked phagocytic properties? More recently H. and M. Westhues ('25) performed clever and painstaking experiments to prove this point. They believed that Permar's inferences, drawn from lung sections that had necessarily been cut in many planes, were "very daring." They attempted to narcotise the phagocytes, but without success. Next they replied to my earlier criticism by using colloidal Elektrokollargol for intratracheal

and Elektroferrol for intravenous injection. No phagocytosis was observed on the part of the capillary endothelium. This would appear to be an important point, were it not for the fact that the endothelium was presumably healthy and would therefore scarcely phagocytose foreign material. They then perfused rabbit lung with normal saline solution to wash the circulation clear, injected 1: 150 India ink into the trachea and suspended the lung in warm saline solution in an incubator for half and hour. The ink was taken up avidly by the dust cells. Reversing the process, they flooded the capillaries with ink and the alveoli with normal saline solution, observing no phagocytosis of the ink by the endothelium or the dust cells. In the first instance no ink was shown to have been phagocytosed by the respiratory epithelium *in situ*, either in the description or illustrations; in the second, although it is stated that there was no phagocytosis of the ink by the endothelium, the colored illustration depicts black granules all through the walls of the alveoli and embedded in the violet tinted cytoplasm of their cells. It is difficult to reconcile this unfortunate discrepancy with the text of the article.

Theories of Mesenchymal Origin of the Dust Cells: So much for the epithelial origin of the dust cell, let us now consider the other side of the argument. When Permar and I published our experimental results we were content to avail ourselves of the evidence at hand; perhaps it was insufficient. My line of deduction was as follows: Cells were found in the alveolar spaces in tuberculous inflammation containing carbon that had been administered intravenously. It appeared as fine particles and coarser clumps in what was interpreted as capillary endothelium which was swollen and which showed mitotic figures in what were supposed to be its lining cells. Therefore the intra-alveolar phagocytes were emigrated cells from the capillary walls. Experimentation with meningeal tuberculosis (Foot,'22) subsequently proved that there was little change in the local capillary endothelium in that case, yet tubercles formed near the vessels and consisted of carbon-marked cells. Similar cells were found within the lumina of the pial vessels, often in mitosis and mingled with the other blood cells. Obviously something was wrong with the earlier theory, which should have applied here as well, or this was a notable exception to the rule. It is possible that what Permar and I interpreted as vascular endothelium in mitosis,

was, in reality, mitosis in monocytes adhering to the capillary walls on the inside.

It is apparent that one can neither prove nor disprove much in this connection unless the process be observed *in vivo*, or many methods be applied simultaneously. M. R. Lewis ('25) has observed carbon-laden monocytes leaving the pulmonary capillaries to enter the alveoli of the lungs of living frogs; she mentions, however, that this animal does not normally possess dust cells. Herzog ('24) has also noted carbon-laden sessile cells in the capillaries of a frog's tongue detaching themselves and floating off into the circulation, or migrating through the vessel walls and wandering to points quite distant therefrom. Of course, these "sessile cells" may have been clinging monocytes and only apparently connected with the capillary endothelium.

That even observations *in vivo* are subject to misinterpretation is shown by the fact that Stilwell ('26) has repeated Herzog's experiments in Maximow's laboratory and has been unable to confirm them. She finds that the vascular endothelium does, indeed, store ink for a time, indulging in what she calls "passive phagocytosis," but this ink is gradually transferred to the perivascular tissue while under direct observation. Furthermore, the ink-laden cells that leave the vessels in the frog's tongue are, according to her views, monocytes; she did not observe any rounding-up of the vascular endothelium to produce monocytes or polyblasts. This is of interest not only because it shows Herzog to be in error, but because it entirely refutes my former claims as to the specificity of ink for vascular endothelium and the argument that the cells seen to migrate from the vessels were therefore detached vascular endothelium. In the face of such observations, made *in vivo* and therefore quite different from deductions derived from the study of fixed tissue, one can only retract one's claims and admit that they are unsound.

Thus we come to a point where the matter seems to require some method other than the mere observation of sections of fixed tissue, be they ever so carefully made, for its solution. Wislocki ('24) has considered the origin of the dust cell to be impossible of detection by this means. He says: "We find it impossible from sectioned material or by the methods employed for its identification, to determine its origin." Employing supravital staining with neutral red and Janus green and dyeing the scrapings of fresh lung from

rabbits and a cat, after a preliminary intravenous injection of Higgins' ink, he found that the various pulmonary elements could be identified and classified. The leucocytes, erythrocytes and monocytes were easily recognized; the dust cells were found to be roughly divisible into three classes: Type (1) large cells with oval nuclei and a variable number of large neutral red vacuoles, Type (2) slightly smaller cells with many carbon granules and a smaller number of red vacuoles, Type (3) large cells which, in addition to a few specks of carbon and the neutral red vacuoles, contained large, greenish, refractile granules arranged about the periphery of the cells. The carbon charge was always in inverse ratio to the number of neutral red vacuoles, the inference being that the cells preferred carbon to neutral red; just as in Westhues' ('22) experiment it was found that they had a greater affinity for carbon than for carmine. The epithelium was often found in sheets or cords, it was partly of the non-ciliated cuboidal and partly of the ciliated cylindrical type. Its cytoplasm was filled with minute red granules. None of the epithelial cells contained carbon. Transitions between "clasmatocytes" (dust cells) of Types 1 and 2 were observed.

Wislocki concludes: "The carbon deposited in the capillaries of the lung is gradually eliminated probably by way of the circulation, the respiratory tract and the lymph channels. After the initial phase of deposition of carbon particles in the lungs, phagocytic cells play a prominent part in its storage and elimination. By studying the fresh, living cells of the lungs in a warm-box, it has been shown that the carbon particles are phagocytosed principally by clasmatocytes. The origin of these cells in the lungs is discussed." As to this point, he says: "Three possibilities suggest themselves. The first is that it (the dust cell) arises by mitosis from the endothelium of the pulmonary capillaries. The second is that it arises in the liver and spleen and is carried by the circulation to lodge in the lungs. The third possibility is that it arises in the stroma of the lungs from clasmatocytes normally present there." In explanation, it may be said that "monocytes" are Aschoff's "blood-histiocytes," the "large mononuclear leucocytes" of clinical parlance; while "clasmatocytes" are the "tissue histiocytes" of Aschoff, "endothelial leucocytes" of Mallory, "polyblasts" of Maximow, etc.

An article by Masugi ('27) appears in the same number of "Ziegler's Beiträge" as does Gross' paper; it describes the tinctorial char-

acteristics of the monocyte and histiocyte when treated supravitally with neutral red and Janus green or stained with other dyes. The beautiful color plates correspond very closely with Wislocki's description of the cells observed in lung scrapings. As Masugi's work was undertaken "at the suggestion and under the direction of" Professor Aschoff, it would seem that Wislocki had satisfactorily complied with the criteria for identifying monocytes and histiocytes as laid down by the latter. This will be of great importance later on in this article.

Fried ('27) has just published a report in which he apparently denies the presence of the alveolar epithelium altogether, but a careful perusal of his paper brings out the fact that he, too, concedes the existence of non-nucleated epithelial plates. According to him, what appears to be respiratory epithelium is, in reality, masses of histiocytes that are attached to the alveolar wall near the angles and which, when stimulated by the presence of foreign material, become detached to give rise to the typical dust cell, or "Herzfehlerzell." His most striking results were obtained in rabbits by injecting large doses of pyrrhol blue in 1 per cent concentration in Ringer's solution intratracheally, either in one massive (acute) dose, or in five smaller (chronic) doses of 5 cc. each. Because the cells attached to the alveolar wall gave a typical picture of vitally stained histiocytes, he believes that they are of mesenchymal and not of epithelial origin. His conclusions are necessarily based upon morphology alone. The epithelium of the bronchi, although drenched in the dye, remained unstained; the vascular endothelium of the pulmonary capillaries was also unstained; he could demonstrate no respiratory epithelium and therefore he disbelieves in its existence. He rules out the monocytes as parent cells because of the total lack of histiocytic proliferation in the other organs. Well founded though these conclusions may be, they leave loop-holes for the critic in so far as they fail to explain the histogenesis of the groups of mural histiocytes, and overlook the possibility of independent propagation of the monocytes while circulating in the blood stream. His failure to demonstrate respiratory epithelium was due to his technic, for it may be easily demonstrated with silver impregnations.

Having surveyed the literature in a fairly comprehensive fashion, one must admit that the subject is still unsettled, as both Gardner ('26) and Sacks ('26) remark in recent reviews on this topic. If there

be a way in which it may be settled, it would appear to lie along the lines of: (*a*) Supravital staining, (*b*) intravital or supravital injection of dyes, (*c*) by the discovery of a specific method for staining the dust cell and its parent cell in fixed tissues. Therefore the work detailed in the remainder of this paper has been undertaken along these three lines. For a more general consideration of the ramifications of the reticulo-endothelial system the reader is referred to reviews by Aschoff ('24), Foot ('25), Gardner ('26), Sabin ('22) and Sacks ('26).

Report of Original Experimental Work

In order to attack this problem from several angles at once, the following experiments were performed. Rabbits were given intravenous and intratracheal injections of various dyes and of milk, the cells that responded in the alveoli were examined *in vivo* in supravital films, the lungs were sectioned after freezing or after embedding in paraffin, and examined from the morphologic standpoint. A specific method for identifying monocytes, histiocytes and dust cells on the one hand and distinguishing them from epithelium or from pleural mesothelium on the other, was fortunately found. Material from scraped human lungs was examined supravitally as soon as permissible postmortem and the sputum from a patient with aortic insufficiency was investigated in supravital films. Lung tissue from similar cases was sectioned in paraffin and studied with routine stains and the specific silver tannate technic. The findings with the latter were checked up with material from fresh human spleen and a case of tuberculous meningitis. Fresh human spleen was also examined in supravital films and the findings confirmed those obtained with the silver technic.

Experiments on Rabbits

Technic (Experiment No. 1). The first experiment combined the technics of Eliot ('26) and Wislocki ('24). Two-tenths gm. of carmine rubrum optimum (Coleman & Bell) were ground in a mortar until fine and stirred with 6 cc. of distilled water, added little by little, until the mixture was smooth. It was centrifugated for ten minutes to throw down the coarser particles, boiled and cooled to body temperature. Four cc. were then injected into the ear vein of

a rabbit and 25 cc. of its heart's blood were withdrawn after a wait of fifteen minutes into a syringe containing 2 cc. of 10 per cent aqueous sodium citrate. After mixing this well, 8 cc. were injected intravenously into each of three rabbits. These next received intratracheal injections of from 3 to 4 cc. of pasteurized milk containing 0.5 per cent of saturated alcoholic Sudan III. The milk was administered slowly through an aspirating needle introduced directly through the skin into the trachea, an influx of air bubbles into the syringe indicating that the needle had penetrated to the lumen. The rabbits stood the injection well; there was transient dyspnea with some regurgitation of milk, but after a minute or two they were on their feet again and quite alert. By the next day they seemed perfectly well.

One rabbit was killed 24 hours, the next 48 hours and the third 90 hours after the injection, by introducing air into the circulation. Necropsy revealed lungs of an almost normal external appearance in the case of the first two rabbits, but section showed some consolidation at the bases, and slices from these sank in water. Nevertheless, the consolidated lung lacked the congestion and granular appearance of the usual pneumonia. The lungs of the third rabbit were in no way grossly remarkable.

As each rabbit was killed, the chest was opened, the lungs and heart removed *in toto* and bits of lung were either squeezed out or scraped off on slides coated with neutral red and Janus green or Nile blue sulphate. Coverslips were sealed over the drops with paraffin-vaseline, a little Ringer's fluid being added if the material was too scanty to cover the whole coverslip, and the preparations were incubated for a few minutes at body temperature before being examined microscopically on a warm stage. The slides were prepared by adding 40 drops of 1 per cent neutral red in absolute alcohol and 30 drops of Janus green in like solution, to 10 cc. of absolute alcohol, flooding clean slides and allowing them to dry. The Nile blue sulphate was used undiluted in 1 per cent solution in absolute alcohol. Before the blood from the donor rabbit was injected into the others, it was examined to determine that there was no free carmine in its plasma.

After these films were completed, the lungs were injected through the trachea with 10 per cent neutral formalin until moderately distended and dropped entire into that fixative. Part of the material

was sectioned on a freezing microtome and part embedded in paraffin and subsequently sectioned at 7 microns.

(Experiment No. 2.) This practically duplicated the first, but five cc. of the carmine suspension were injected intravenously into the donor rabbit and its blood was withdrawn five, instead of fifteen minutes later. Only two rabbits received this blood intravenously and only 1 cc. of milk (this time diluted one-half with Ringer's fluid) was injected into their tracheae. The supravital examinations were omitted in this case and the lungs were removed *in toto* 24 and 48 hours after they had been injected with the milk. In neither case was there anything abnormal to be noted, the organs being of normal color, consistence and general appearance.

Donor Rabbits: The donor for the first experiment died about a week after giving its blood. No gross evidence for its death was manifest at necropsy. Its lungs were removed and injected with neutral formalin, as in the other cases; its spleen was also fixed for microscopic examination.

The donor for the second experiment was killed by air-embolus 96 hours after the bleeding and its lungs (which appeared in no way abnormal, although in this case the donor rabbit had also received a milk injection) were fixed as above. Bits of spleen and liver were also secured for examination as to the distribution of the carmine.

Purpose of the Experiments

By introducing "marked monocytes" from another animal, it was hoped that the blood origin of the intra-alveolar phagocytes might be proved or disproved; if these contained carmine it is obvious that they would most probably represent marked cells from the donor animal — unless polymorphonuclear leucocytes should have emigrated and been engulfed by the dust cells, in which case uncertainty would result. The milk was used as a mild irritant, to excite the emigration of macrophages into the alveolar sacs; incidentally, the neutral fat would serve as a dye if it retained the Sudan III, if not, use could still be made of it by staining with more of that dye or using Nile blue sulphate. Ballon and Ballon ('27) have used this method for tracing the fate of lipiodol in the lung. The purpose of the supravital stains was to determine the behavior of the dust cells, the epithelium, vascular endothelium and mesothelium toward

these stains. Nile blue sulphate would also act as a fat stain and indicate whether the neutral fat underwent chemical changes after becoming incorporated in the cells.

Results in Supravitally Stained Films

These confirmed Wislocki's findings in every particular, his three types of "clasmatocytes" were recognized and their striking similarity to Masugi's illustrations was at once noted. The results with Nile blue sulphate were as satisfactory as those with neutral red; this dye gives more rapid impregnation of the granules and the pictures are sharper. Reviewing the types of cells noted in both stains, but omitting a description of the neutral red pictures, — as Wislocki has already covered this, — we find the following:

Dust Cells: These were large, rounded or ovoid cells with numerous dark blue to greenish granules which varied not only in color, but also in size and shape. There were fine, sharply stained granules and large, variably stained vacuoles which sometimes contained a speck or two of dust. Some of these cells showed uniform dark blue granules with a pale, yellowish, unstained nuclear space; others showed the variable characteristics noted for dust cells in general, while a third type — the largest of the three, contained large, polyhedral, refractile pieces of yellowish or brownish material as well, sometimes almost as large as erythrocytes. Frequently there were large vacuoles in the third type that contained either erythrocytes or drops of neutral fat, which stained rose with the Nile blue sulphate. The mitochondria seen in the neutral red-Janus green technic were not prominent in this case. Sometimes one occasionally observed carmine-colored granules, but as they were also present in films from controls that had received no carmine they probably represent metachromatic staining. Attraction-spheres were not prominent. The carmine injected in the first experiment could not be found in the blood cells of the animals that received the transfused blood. This is not readily explained, for the only differences in the technic, as compared with Eliot's, were the use of sodium citrate instead of heparin and a slightly longer delay in withdrawing the blood. The second experiment demonstrated that this delay could have made little difference in the results.

Monocytes: Small monocytes were frequently encountered in the stained films; a little larger than polymorphonuclears, they were

usually filled with blue granules, uniform in size and brilliantly stained, with the untinted nucleus crowded to the periphery. A large proportion of these showed a cluster of granules in the concavity of the reniform nucleus, with a more thinly distributed line of granules extending like the horns of a crescent to the nuclear poles; a comparatively unstained zone separated them from the cell periphery (Figs. 1 and 2). The appearance of these cells, when stained supravitally with neutral red and after fixation with Sudan III and silver tannate, is shown in Figs. 4, 5 and 6. Figure 3 shows a monocyte with a phagocytosed fat droplet, transitional between Figs. 1 and 2 and Fig. 7, which represents a "Type 3" dust cell. Figure 8 is a similar transitional type impregnated with silver tannate and containing a fat vacuole.

Polymorphonuclears: These stained a diffuse light blue and showed yellowish granules, with a few that were blue.

Lymphocytes: They took on an even, diffuse light blue color though unstained as to nucleus and granules.

Vascular Endothelium: Bits of capillaries were included in the scrapings (Fig. 10), with a few erythrocytes in their lumina. Nothing indicated that they stained specifically, nor were the fibroblasts at all granular.

Epithelial Cells: Three types were recognized:

(1) Large, flat or slightly curled plates with no nucleus or merely a shadow of one (Fig. 9).

(2) Groups of interlocking, flanged cells, some of them showing denser nuclear shadows and resembling the descriptions of Bremer ('04), Ogawa ('20) and Stewart ('23).

(3) Non-ciliated cuboidal, or ciliated cylindrical cells from the bronchial mucosa.

None of these cells stained deeply, the larger cells showed a diffuse light blue, but no granules (very fine granules were seen with neutral red), while the small bronchial cells either stained not at all, or only faintly with an occasional minute granule of Nile blue sulphate. Some of the groups showed a drop or two of rose-colored neutral fat in, or on, their cytoplasm. Branching, Y-shaped streaks were observed in the large epithelial plates, corresponding to Stewart's pictures of epithelial mitochondria. Sometimes, where there were extensive sheets of epithelium which had separated from the alveoli, one might observe vitally-stained, rounded cells lying

in small fenestra in the membrane; these were probably "septum cells" seen from above.

Mesothelium: Sheets of pleural tissue were quite unstained with Nile blue sulphate, although very fine, closely arranged and refractile granules which stained faintly with neutral red could be observed in their cytoplasm.

Free Fat: This was present in the form of larger or smaller drops of light orange or rose, according to the stain. Apparently most of the Sudan III in the milk had disappeared.

Frozen Sections; Sudan III Stain

(*First Experiment*)

These showed that the dyed milk injection had caused a mild lobular pneumonia in which the exudate was composed of polymorphonuclear leucocytes and macrophages in about equal numbers; the greatest reaction was seen in the lung of the two-day rabbit. After four days the lungs had practically returned to normal, but there was still a large number of dust cells in the alveoli. As we are interested chiefly in the fat in these sections, let us confine our attention to that substance. It lay free in the air-sacs at first and was then taken up by the phagocytes, the epithelium being merely dusted with fine particles. The large cells protruding from the alveolar septa (considered to be epithelium by Aschoff, Gross and Westhues, and histiocytes by Kiyono, Fried and Gardner), contained much fat. In the frozen sections there was little, if any clue as to the origin of these cells. The monocytes in the capillaries, the polymorphonuclears to a lesser degree and the vascular endothelium all showed intracellular fat and there appeared to be some of it free in the blood plasma, although this may have been scattered out in the process of sectioning. Most of it was contained in dust cells and monocytes. Occasionally globules were found between the bronchial epithelial cells, but their cytoplasm was practically free from Sudanophil material, although diffusely "rusted" by the stain. There was a moderately heavy deposition of fat in the peribronchial lymph nodes, some within phagocytes and some apparently free.

The areas of bronchopneumonia in the first two rabbits showed such distortion of the normal pulmonary architecture that very little could be judged as to what had taken place; apparently the walls

of the alveoli had become thickened by engorgement of the capillaries and proliferation of the stroma, and exudate had filled the air-sacs here and there. There were fields of large, pale cells liberally dotted with fat, closely resembling those in Fried's photomicrographs, but there was little evidence to show whence they came. These might have been histiocytes, proliferated adventitial cells, thickened capillary endothelium or migrated monocytes — that they were epithelium, however, was most improbable as they lay outside of the air sacs, rather than within them.

The only assistance obtained from the use of Sudan III, then, is the fact that it is taken up by the phagocytes in a manner that stains them in the same way that either neutral red or Nile blue sulphate does. The granules, vacuoles and foreign material are found to correspond accurately in all three methods, therefore there must be a striking similarity in the chemistry of these stains within the cytoplasm.

Paraffin Sections

(*First Experiment*)

These differed very little from the frozen sections. As the fat had been extracted by the chloroform, the pictures were somewhat less complicated. No carmine could be definitely identified, although many leucocytes and dust cells contained minute granules that appeared to give off a reddish luster under the oil-immersion lens. The experiment, then, completely failed to corroborate Eliot's observations.

Silver Tannate Impregnations of Paraffin Sections: (First experiment.) As cell granules were well demonstrated by silver tannate impregnations used in experimenting with the Rio de Hortega technic (Foot, '27), it was supposed that this method might aid in solving the dust cell problem. As a result, a very satisfactory means of identifying monocytes and polyblasts was discovered. The sections impregnated with silver tannate showed that the dust cells became reddish brown, with brownish black to sepia granules that corresponded in every way with those observed in the supravital films and the Sudan III sections. Furthermore, these granules were usually grouped in rosettes or balls as in typical monocytes. No other cells in these sections except the polymorphonuclears showed similar characteristics; these had similar, but rather finer granules,

and in the fat-treated lungs they tended to take on a somewhat reddish tinge.

Here, then, is a means of impregnating dust cells specifically. As there was a possibility that this depended upon the presence of ingested fat, lungs from six other rabbits were sectioned and impregnated in the same way, invariably showing the same thing although presumably fat-free. The dust cells, monocytes and polymorphonuclears were the only cells that became specifically impregnated. By combining supravital intratracheal staining with neutral red, Niagara blue 3b or Nile blue sulphate injected at the time of death from air-embolism and incubated *in situ* in the dead rabbit for fifteen minutes, as recommended by Gardner ('27), slight variations in the color reactions of the impregnation were effected, but the granular impregnation remained the same. As the fixation was ordinary neutral formalin and the routine paraffin technic was used, instead of Gardner's more elaborate procedure which requires an adjustment of the pH of the fixative necessitating special color indicators or a potentiometer, the results with neutral red were inferior to his. The cells showed the typical granular stain seen in supravital films, however, but the bright red of these had paled to a dull brick-red.

In order further to check up on the specificity of the silver tannate method, smears of rabbits' blood were made, fixed in neutral 10 per cent formalin and impregnated. The monocytes and polymorphonuclears showed the specific dark brown cytoplasm and sepia granules seen in the sections, although the monocytes tended to be paler in the smears. The lymphocytes were pale and showed no granular stain, their cytoplasm was almost colorless and their nuclei stood out distinctly, as with iron hematoxylin. The erythrocytes were either slightly and diffusely brownish, or where they had dried and laked somewhat, showed blackish reticulation. This reticulation was observed in some of the sections also.

Observations in Silver Tannate Sections: In the sections from the first milk-injection experiment, monocytes were found crowding the capillaries, undergoing karyokinesis while within their lumina, and dividing within the alveoli after emerging from the vessels. A monocyte is shown in a large vein in Fig. 11. Careful scrutiny of all the slides failed to demonstrate any coarse granules in the epithelium, mesothelium or vascular endothelium. The large cells that

are attached to the alveolar septa appeared in the rôle of greatly swollen, clinging monocytes; smaller monocytes might be seen congregated in the capillaries and apparently emerging therefrom (Fig. 12). There was a general dotting of the various pulmonary elements by a light silver precipitate, but this was totally different from the sharply defined and definitely grouped granules in the monocytes or dust cells (Fig. 13). In properly impregnated sections the epithelium of the alveoli could be observed as it lined the extremity of an air-sac and one could discern the epithelial cells and non-nucleated plates, and the capillaries and reticulum beneath them (Fig. 14). The latter was an immense help in keeping the topography of the lung clear in the infiltrated areas. The epithelium could be better brought out in the sections where neutral red had been injected at the time of death. It was seen to correspond accurately with the descriptions of Ogawa and Stewart, except that my preparations showed fenestrations in the epithelial membrane, often occupied by intercalated dust cells. Of course, the empty fenestra might have been fixation artifacts, but the presence of dust cells in some of them, both in these sections and the unfixed scrapings observed *in vivo*, makes this quite unlikely.

Where the alveolar walls had become much thickened in the areas of lobular pneumonia, one noticed two things: The capillaries were distended with monocytes and polymorphonuclears and their endothelium had become vague and merged with the stroma, so that wide fields of pale cells might be observed, with here and there a monocyte lying in the tissue spaces. The cells of these fields resembled vascular endothelium, adventitial or connective tissue cells, — pale, swollen, with ovoid and vesicular nuclei and vacuolated, reticulated cytoplasm. They showed no definite granules other than an occasional blackish grain that might have been precipitate (Fig. 15). On more than one occasion dust cells were observed to be enveloped in veil-like epithelial plates, as though these had been lifted off by them and carried into the alveolar space, where they remained folded about the cell that had detached them.

Findings in Second Rabbit Experiment

Attention was focussed upon paraffin sections stained with hematoxylin alone or impregnated with silver tannate. The observations tallied in the main with those of the preceding experiment, but the

mixture of equal parts of pasteurized milk and Ringer's solution, injected in much smaller quantities, caused only a local reaction in the bronchi. There was no pneumonia. No carmine-marked cells were found in any of the sections, it seemed as though none had been transferred from the donor rabbit, despite the fact that it had a larger intravenous injection of carmine suspension than did the first donor.

Findings in Donor Rabbits

The donor rabbit of the first experiment showed relatively little carmine in the lungs and a good deal in the spleen. In the former it was contained in monocytes and polymorphonuclears within the capillaries and very occasionally within a dust cell in which it appeared as a group of carmine particles that occupied about as much room as would an erythrocyte. In the spleen the carmine was in large macrophages which, owing to the fact that the rabbit was found some time after its actual death, also contained the oxydase granules that one finds in somewhat "spoiled" formalin-fixed tissue. These were brownish black and corresponded in their arrangement with the vital granules seen in supravital, Sudan III and silver preparations. These were also present in the monocytes and polymorphonuclears of the capillary sinuses and larger vessels, where more or less carmine was also encountered.

Two points are brought out in this case: The spleen had apparently acquired most of the injected carmine. Certainly it contained more than the lungs, and the fact that dust cells were found in the air-sacs, laden with carmine that had been introduced intravenously, strongly indicated that this particulate material had been carried out of the vessel within the cytoplasm of emigrating monocytes, rather than transferred from the circulation to intra-alveolar dust cells. If the latter hypothesis were true, the carmine would have to traverse the vascular endothelium, stroma and alveolar epithelium. Furthermore, no carmine was found free in the alveolar spaces.

The donor rabbit in the second experiment, killed by air-embolism four days after bleeding, showed some carmine in macrophages in the spleen and a trace in the Kupffer cells of the liver. None was found in the lung. Although this animal had some milk injected into the trachea, the lungs were practically normal.

Silver tannate impregnations of the liver and spleen showed the

specific granular stain in the Kupffer cells of the former and in monocytes circulating in the sinusoids; the spleen was sprinkled with monocytes, also showing the specific granules. These findings further strengthen the supposition that this method marks monocytes specifically, and they point out the fact that the Kupffer cell is, indeed, different from ordinary endothelium. It takes a granular impregnation quite similar to that seen in the monocyte, which apparently indicates a close relationship between the two. Are Kupffer cells, for instance, merely monocytes anchored to the sinusoidal endothelium by one or more pseudopods?

Examination of Human Material

Supravital Films of Lung Scrapings: Scrapings made from lungs in cases of chronic passive congestion were treated exactly like those from the rabbit lungs; the cases chosen for examination were necropsied as soon after death as permissible. In lungs removed as long as eight and fourteen hours postmortem, the results resembled in every detail those obtained in the case of rabbits. The cells were still viable in both instances and were observed on a warm-stage, the staining of the nuclei being considered a criterion of cell death. The same types of epithelium, stroma, blood cells and dust cells were observed; the latter were plainly marked with the carbon that abounds in Cincinnati atmosphere.

Supravital Films of Sputum: Fresh sputum from a case of cardiac decompensation was obtained from the wards on two occasions and examined by the supravital method; again the results were quite similar, although the copious and tenacious mucus interfered rather noticeably with the efficacy of the neutral red. Nile blue sulphate, however, still gave good results, and the check-up on the observations of necropsy material was perfectly satisfactory.

Paraffin Sections from Human Lung: Specimens of lungs showing chronic passive congestion were sectioned and stained with hematoxylin and eosin as a control, and the silver tannate impregnation was used for critical observation. They showed, even more strikingly than the rabbit lung, the specific character of this impregnation for dust cells. These were very dark, filled with small, uniform black granules and they stood out in bold relief in comparison with the desquamated epithelial cells whose cytoplasm showed no granules and was rather vacuolated and violet-gray. This is well shown

in Fig. 16. Here again, one found the epithelial plates wrapped about the dust cells. Comparing the hematoxylin and eosin controls with the silver impregnations, one noted that the former showed dust cells heavily laden with hemosiderin and carbon ("Herzfehlerzellen"), but totally lacking the smaller, uniform black granules that were so prominent in the silver impregnations (Fig. 17). Another case of chronic passive pulmonary congestion with infarcts was examined and the granular impregnation of the dust cells was far less striking; this was in material that had been fixed some time postmortem and proves that one should be sure of the freshness of one's material before drawing conclusions adverse to this technic. It has been found to be quite worthless in brain tissue that has remained unfixed for a day or more. Even shorter periods are unfavorable to the specificity of the stain in warm weather.

Spleen and Brain: Paraffin sections from a human spleen and from the meninges in a case of tuberculous meningitis show that the monocytes, polymorphonuclears and polyblasts in these become impregnated exactly as in the case of lung tissue. The splenic endothelium of the capillary sinuses differs absolutely in its staining properties from these cells. The reticulo-endothelium is so vacuolated, so intimately associated with fibrils, and its granules so indefinite, that it also differs from them to a certain extent.

Silver Tannate Technic *

Cut thin paraffin sections from formalin or Zenker-fixed material and remove the paraffin in the usual way. If Zenker's fluid has been used, remove the mercury by treating the sections for five minutes in mahogany-brown, alcoholic iodin solution. Bleach in 5 per cent aqueous sodium thiosulphate. Wash. Remove the chromium salts by five minutes treatment in 0.25 per cent potassium permanganate and ten minutes in 5 per cent oxalic acid, washing between solutions. Wash and re-wash in distilled water. Mordant for fifteen minutes in a solution of 0.15 per cent pure tannic acid, 3 per cent ammonium bromid and 10 per cent neutral formalin; this should be done in an incubator, first heating the mordant to 55° C. Treat for thirty

* It is advisable to summarize this procedure here, although it is described in full elsewhere (Foot, '27).

seconds with three drops of strong ammonia to 100 cc. of distilled water, while the sections are still warm. Impregnate for five minutes in silver-ammonium oxid prepared as follows: To 10 cc. of 1 per cent silver nitrate add one drop of 40 per cent sodium or potassium hydroxid, dissolve the precipitate in five drops of strong ammonia (which should leave a few grains still out of solution), dilute up to 200 cc. with distilled water. Use two baths if the first becomes turbid after two or three minutes. Wash in distilled water and "reduce" for two minutes in 20 per cent neutral formalin. Wash at the tap. "Tone" for two minutes in a 1:500 solution of Merck's "brown, acid" gold chlorid, in which 0.5 per cent bichloride of mercury has been dissolved with the aid of heat. Wash at the tap and fix in 5 per cent sodium thiosulphate (Hypo) for two minutes. Wash and dehydrate in the usual manner with alcohol of increasing percentages, xylol, and mount in Canada balsam.

If the sections be too dark, they may be lightened by immersion in strong potassium cyanid solution (aqueous), but it is better to run through a new set of sections and increase the strength of the ammonia wash, used after the mordant, to ten drops instead of three to 100 cc. of distilled water. Tissues vary a good deal and one must do a certain amount of experimentation on each batch of slides in order to produce the best results. The depth of impregnation may be largely controlled by the strength of the ammonia wash — a weak wash producing dark sections, a strong one lighter impregnation.

Discussion

We come, finally, to a consideration of this subject as a whole in the light of the evidence of others which is, perhaps, somewhat intensified by the additional data supplied by the experiments just described. The easiest way to undertake this task will be to set down *seriatim* the various hypothetical sources of the dust cell and to discuss each in turn.

Epithelial Origin

As the arguments in favor of this have been set forth at the beginning of the paper, let us consider those opposed to it. There are several valid reasons for rejecting the epithelial theory.

1. Supravital staining with neutral red and Nile blue sulphate and the apparently specific silver tannate impregnation not only

fail to demonstrate any similarity between epithelium, whether alveolar or bronchial, and the dust cell, but actually separate them into two distinctly morphologically unrelated groups.

2. Dust cells often appear in the lymphatics and lymph nodes of the lung laden with carbon, tubercle bacilli, dyes, fat or other material that has come in through the trachea or circulation. There they remain. Permar ('23), Haythorn ('13), Sewell ('18) and Foot ('20) have all reported this phenomenon. Is it more likely that these are cells of mesenchymal origin, or strangely metamorphosed epithelium? Sewell manifested a great deal of difficulty in reconciling the latter view with his findings and his explanation was not, even then, at all convincing. He was forced to regard these as renegade epithelial cells that had become transformed into leucocytes.

3. Macrophages or polyblasts are found in large numbers in a variety of processes in epithelial organs, but apparently it is only in the case of the lung that their presence is ascribed to epithelial proliferation and desquamation.

4. It has been proved that the epithelioid cells of hepatic tuberculosis are formed directly from the Kupffer cells (Evans, Bowman and Winternitz, '14; Goldman, '09, '12; Oppenheimer, '08 and others. To-day no one would consider the bile duct or hepatic epithelium as a source of epithelioid cells in tuberculosis of that organ. As the Kupffer cell appears to be somewhat different from the ordinary vascular endothelium, its participation in the formation of tubercles may be misleading to those who maintain that the vascular endothelium produces the macrophages, as I once believed.

5. Tubercles identical with these arise in the spleen and lymphoid tissue where phagocytes in every way similar to dust cells abound and where there is not epithelium to produce them.

6. Typical multinucleated syncytia, or giant cells, are formed from the dust cells in the alveoli; this is not characteristic of epithelium, which forms multinucleated cells usually under neoplastic conditions, but these differ materially from the typical foreign body giant cell.

7. Stewart ('23) describes the mitochondria of alveolar epithelium, when stained by the Altmann method, as rod-like, often branching or Y-shaped. Such structures may be seen in the epithelial cells in supravital films of scraped lung. I have pictured them in Figure 9. Stewart also shows a mature epithelial cell "about

to desquamate" into the alveolus; the striking change in its granules is at once apparent; instead of rod-like mitochondria, we see spheroidal granules of varying size. The cell corresponds accurately with a swollen monocyte or dust cell; was he not picturing one of these in this instance? No Y-shaped or rod-like mitochondria are seen in the dust cells, which differentiates them sharply from epithelium.

8. Finally, the arguments of the supporters of the epithelial origin of the dust cells are based exclusively on morphologic similarity, chiefly in hematoxylin and eosin preparations or similar sections. This similarity, under these circumstances, is certainly striking; is it, however, sufficient evidence to advance in the face of that of the proponents of the other theories, whose data are the result of observations on a variety of stains and technics?

If we put any faith whatever in our conceptions of tissue specificity, the idea that the pulmonary epithelium is alone capable of producing cells which, to all intents and purposes, not only resemble, but actually become indistinguishable from mesenchymal derivatives (when observed by a number of vital, supravital and fixed-tissue methods) is, to say the least, irrational. Were it established beyond a doubt that the epithelium could become transformed into connective tissue or adult mesenchyma — and *vice versa*, we might accept such an hypothesis with complacency.

Monocytic Origin

The interesting work of the Lewises ('23, to '25), Wislocki's ('24) findings and Maximow's ('26) long series of investigations, as well as the experiments detailed in this paper all point very strongly to the monocytic origin of the dust cell. Cunningham, Sabin and Doan distinguish between monocytes and clasmatocytes on a technical basis, depending upon the respective supravital staining characteristics. That this distinction is entirely warranted is still disputed by authors like Masugi, who either regard these types as phases of the same cell or take issue with the interpretation of their origin. Masugi considers that there are two types, monocytes ("Bluthistiozyten") and histiocytes proper; the Lewises regard these as different forms of the same cell; Maximow ('26) agrees with Masugi's interpretation in substance, but takes a broader view of the matter.

Personal observation indicates that the finding of typical rosettes, which should mark the monocyte as such, is more or less a matter of chance; they may or may not be present. In fixed tissue they seem to occur rather more regularly than in fresh films. Be this as it may, it seems that the weight of opinion is tipping the scale in the favor of the monocyte as the parent of the dust cell.

A very telling point in favor of the monocytic origin is the great rapidity with which these cells appear in the alveoli. The Germans have used this as proof of their epithelial origin, as desquamation could readily account for such a phenomenon, but it does not account for the radical difference in morphology between epithelium and dust cells when properly stained. Migration of monocytes from the capillaries is not open to this criticism, for not only do they exhibit the same general morphology, but they will (if kept alive *in vitro*) actually become indistinguishable from dust cells. This has been noted by Carrell and Ebeling ('22), by the Lewises, by Gardner and in my latest experiments. Mitosis may be observed in monocytes within pulmonary capillaries and in dust cells, which would add to their rapidity of production and, although not entirely accounting for it, would indicate that two closely related types were multiplying in response to the same stimulus.

The origin of the monocyte is for the embryologist to establish; once in existence, however, it seems that this cell is capable of independent self-perpetuation, without drawing on any particular organ or tissue reservoir to repair the inroads of an inflammatory process on the supply at hand. Removal of the spleen does not lessen the supply of monocytes in any way (Foot, '23) and the hypothesis that they are the progeny of the extremely specialized endothelium of the venous sinuses of that organ is contradicted by the striking difference in the morphology of the two types and, even more so, by the fact that splenic sinus cells do not stain at all in supravital films. This I have just determined by experiment on fresh human spleen.

For years Maximow has been championing the lymphocytic origin of some of the "polyblasts" in the organism. His "polyblast" is essentially the same as the macrophage, clasmatocyte or endothelial leucocyte. His theories have been sharply criticised and I ('25) have been one of the critics. He has recently published experiments ('27) that effectually disarm this criticism, as he reports

the observation of a transformation of lymphocytes in tissue cultures into polyblasts and fibroblasts, while alive and growing. His pupil, Bloom ('27), reports the transformation of lymphocytes, taken from the "water-clear" lymph of the thoracic duct of rabbits, into polyblasts. It is indeed difficult to remain skeptical in the face of this evidence, therefore we must add the circulating lymphocyte to the possible sources of monocytes and, through these, of dust cells.

Histiocytic Origin

That the histiocytes, or reticulo-endothelial cells of the pulmonary stroma share in the production of dust cells cannot be denied, but there is reason to believe that they play a subsidiary rôle. These cells do not become as sharply impregnated with silver tannate as do the dust cells, whereas the monocytes do. This does not prove that they are unassociated with dust cells, however, for they are in a different medium, more or less fixed and possibly correspond to the cells that spread out over the surface of the glass in tissue cultures or films. They were much increased in the experiments with milk injection and they contained much fat, hence they cannot be excluded as possible parents of dust cells.

Vascular Endothelium

That this tissue produces dust cells seems unlikely. The vascular endothelium does not stain in the same way and it appears to play an entirely passive part in these experiments. Where the reaction to the fat was most intense, the capillary walls seemed to be thickened, but as they were thronged with leucocytes and as the histiocytes and fibroblasts of the stroma were also more numerous, it was very difficult to ascribe to them any importance in the production of free phagocytes. While loth to retract my original claims concerning the rôle of the capillary endothelium in such conditions until convinced that they had become untenable, seven years of further investigation, a critical study of the experience of others, and the results of my recent study of these have all indicated that the time has come to admit that the origin of these phagocytes is more likely to be found in the blood, rather than in the capillary endothelium. In tuberculosis, measles (Mallory and Medlar, '20), typhus (Wolbach, Todd and Palfrey, '22), Rocky

Mountain spotted fever (Wolbach, '19) and a number of other diseases, there is such manifest proliferation of the vascular endothelium that it might be construed as productive of epithelioid cells and phagocytes, but one could consider this change to be limited to the vessel wall and interpret the wandering phagocytes as emigrated monocytes, or histiocytes. Gardner ('26) points out an inherent weakness in the arguments of Permar and myself, when he says that we admit the ingestion of carbon by the monocytes and yet interpret them as being derived from the carbon-laden vascular endothelium. "But this method of demonstration," he continues, "is subject to the objection that within the vessels there are at least two types of cells which take up the vital stain — the endothelial lining cell and also the circulating white blood corpuscles." This is quite true; like McJunkin ('18, '19) I considered that the monocyte was produced by vascular endothelium, but the matter has taken a different turn as data have accumulated to give evidence to the contrary. Moreover, the vascular endothelium is a tissue that will bear further study, — it is not too well understood at the present time.

Lymphocytic Origin

The theory that pulmonary phagocytes, as "polyblasts," are derived from lymphocytes would, at first glance, seem to be susceptible to the same criticism as the epithelium or vascular endothelium. The lymphocytes do not stain in the same way, either vitally, supravitally, or after silver impregnation, but Maximow's and Bloom's observations point too strongly to this possibility to permit its being lightly dismissed. We have no evidence that the lymphocytes become transformed into dust cells in the alveoli, but we cannot deny that they might become converted into monocytes in the circulation and thus enter the alveoli in a new guise and produce dust cells.

Summary

The wealth of evidence adduced from the literature and the experiments here described go far toward proving that the most likely origin of the pulmonary dust cell is from the blood stream and, more specifically, the monocytes thereof. That various reticulo-endothelial elements, such as the supporting cells of the lymphoid tissue of the lung, the adventitial cells of its vessels or even lymphocytes

themselves, share to a degree in the production of dust cells cannot be denied; that they play the chief part in this production, however, seems improbable. That the vascular endothelium gives rise to dust cells under ordinary conditions seems entirely unlikely, in the face of evidence accumulated during the past decade. In view of this fact it will be necessary to readjust our theories of inflammation so that they may more nearly conform with those of Metschnikoff and the Lewises on the one hand and of Maximow on the other. This *volte face* on my part is made only after due deliberation and in the face of what seems to me to be overwhelming evidence.

Conclusions

1. The dust cells or "Herzfehlerzell" are probably larger forms of monocytes or blood histiocytes. While the tissue histiocytes may play some part in their production, it is more likely that their ranks are recruited from the circulating monocytes of the bloodstream. The origin of these is discussed.

2. The alveolar epithelium of the lung does not produce dust cells in so far as can be ascertained; it possesses totally different affinities for silver salts and can, by means of silver tannate impregnation, be readily recognized and differentiated from the alveolar macrophages, which appear to be of mesenchymal, rather than of endodermal origin. Furthermore, as has already been pointed out by other investigators, the reactions of these groups to supravital stains are equally divergent.

3. The assumption that there is no nucleated "respiratory epithelium" does not appear to be warranted, for sections supravitally stained with neutral red and counter-impregnated with silver tannate show pictures in every way similar to those drawn by anatomic investigators. As the refractive index of alveolar epithelium is very close to that of glass, it is imperative that some procedure be used that will render it artificially visible, or that the light in the condenser be arranged so as to utilize the very slightly higher refractive index of the epithelium, otherwise it will escape notice.

The efficient assistance of Mr. J. B. Homan, of our Department of Medical Art, in taking these photomicrographs is hereby gratefully acknowledged. That of Mr. Marvin C. Mènard, in the animal experiments, is also appreciated.

REFERENCES

Aschoff, L. The reticulo-endothelial system. Lectures on Pathology, New York, 1924.

Ballon, D. H., and Ballon, H. C. The effect of the injection of lipiodol and the rate of its disappearance in normal and diseased lungs. *Canad. M. A. J.*, 1927, xvii, 410.

Baumgarten, v. Ueber die histologische Differentialdiagnose zwischen tuberkulöser u. gummöser Orchitis. *Verhandl. d. deutsch. path. Gesellsch.*, 1901, iii, 107.

Bloom, W. Transformation of lymphocytes of thoracic duct into polyblasts (macrophages) in tissue culture. *Proc. Soc. Exper. Biol. & Med.*, 1927, xxiv, 567.

Bremer, J. L. On the lung of the opossum. *Am. J. Anat.*, 1904, iii, 67.

Carrel, A., and Ebeling, A. H. Pure cultures of large mononuclear leucocytes. *J. Exper. Med.*, 1922, xxxvi, 365.

Cunningham, R. S., Sabin, F. R., and Doan, C. A. The differentiation of two distinct types of phagocytic cells in the spleen of the rabbit. *Proc. Soc. Exper. Biol. & Med.*, 1924, xxi, 326.

Cunningham, R. S., Sabin, F. R., and Doan, C. A. The development of leucocytes, lymphocytes and monocytes from a specific stem-cell in adult tissues. *Contrib. Embryol.*, Carnegie Institution of Washington, 1925, xvi, no. 84, 227.

Cunningham, R. S., Sabin, F. R., Doan, C. A., and Kindwall, J. A. The rôle of the monocyte in tuberculosis. *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 231.

Eliot, C. The origin of the phagocytic cells in the rabbit. *Bull. Johns Hopkins Hosp.*, 1926, xxxix, 149.

Evans, H. M., Bowman, F. B., and Winternitz, M. C. An experimental study of the histogenesis of the miliary tubercle in vitally stained rabbits. *J. Exper. Med.*, 1914, xix, 283.

Foot, N. C. The endothelium in experimental pulmonary tuberculosis. *J. Exper. Med.*, 1920, xxxii, 533.

Foot, N. C. The endothelium in experimental general miliary tuberculosis in rabbits. *J. Exper. Med.*, 1921, xxxiii, 271.

Foot, N. C. The endothelial response in experimental tuberculous meningoencephalitis. *J. Exper. Med.*, 1922, xxvi, 607.

Foot, N. C. Changes in the distribution of colloidal carbon noted in the lungs of rabbits following splenectomy. *J. Exper. Med.*, 1923, xxxvii, 139.

Foot, N. C. The endothelial phagocyte, a critical review. *Anat. Record*, 1925, xxx, 15.

Foot, N. C. A silver tannate technic for paraffin sections from the central nervous system. *Arch. Path. & Lab. Med.*, 1927. iv, 36.

Fried, B. M. The origin of the histiocytes (macrophages) in the lungs. *Arch. Path. & Lab. Med.*, 1927, iii, 751.

Gardner, L. U. Tuberculosis-Bacteriology, Pathology and Laboratory Diagnosis. Baldwin, Petroff and Gardner. Philadelphia, 1926.

Gardner, L. U. Preserving supravital staining with neutral red in paraffin sections of the lung. *Proc. Soc. Exper. Biol. & Med.*, 1927, xxiv, 646.

Goldmann, E. E. Die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der vitalen Färbung. Th. 1. *Beitr. z. klin. Chir.*, 1909, lxiv, 192.

Goldmann, E. E. Die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der vitalen Färbung. Th. 2. *Beitr. z. klin Chir.*, 1912, lxxviii, 1.

Gross, F. Ueber die alveoläre Reaktion der Lunge gegenüber Russ, Quartzstaub und Phthisebazillen und die hier herrschenden Lokalisationsgesetze. *Beitr. z. path. Anat. u. z. allg. Path.*, 1927, lxxvi, 374.

Haythorn, S. R. Some histological evidence of the disease importance of pulmonary anthracosis. *J. Med. Res.*, 1913, xxix, 259.

Herxheimer, G. Ueber die Wirkungsweise des Tuberkelbacillus bei experimenteller Lungentuberkulose. *Beitr. z. path. Anat. u. z. allg. Path.*, 1903, xxxiii, 363.

Herzog, F. Ueber Capillarendothelien als Wanderzellen. *Klin. Wchnschr.*, 1924, iii, 535.

Herzog, F. Endothelien der Froschzunge als Phagocyten und Wanderzellen. *Ztschr. f. d. ges. exper. Med.*, 1924, xliii, 79.

Kageyama, S. Ueber die frühzeitigen Reaktionen des retikuloendothelialen Systems bei phthisisch-tuberkulöser Infektion. *Beitr. z. path. Anat. u. z. allg. Path.*, 1925, lxxiv, 356.

Kiyono, K. Die vitale Karminspeicherung. Jena, 1914.

Kiyono, K. Zur Frage der histiozytären Blutzellen. *Folia haemat.*, 1914, xviii, 149.

Lang, F. J. The reaction of lung tissue to tuberculous infection in vitro. *J. Infect. Dis.*, 1925, xxxvii, 430.

Lewis, M. R. The formation of macrophages, epithelioid cells and giant cells from leucocytes in incubated blood. *Am. J. Path.*, 1925, i, 91.

Lewis, M. R. Origin of the phagocytic cells of the lung of the frog. *Bull. Johns Hopkins Hosp.*, 1925, xxxvi, 361.

Lewis, M. R. The resemblance between Kupffer cells and the mononuclear leucocyte of the frog studied in vitro (abst.). *Anat. Record*, 1925, xxix, 390.

Lewis, M. R., and Lewis, W. H. The transformation of white blood cells into clasmatocytes (macrophages, epithelioid cells and giant cells). *J. A. M. A.*, 1925, lxxxiv, 798.

McJunkin, F. A. A simple technic for the demonstration of a phagocytic mononuclear cell in peripheral blood. *Arch. Int. Med.*, 1918, xxi, 59.

McJunkin, F. A. The origin of phagocytic mononuclear cells of the peripheral blood. *Am. J. Anat.*, 1919, xxv, 27.

Mallory, F. B., and Medlar, E. M., The skin lesions in measles. *J. Med. Res.*, 1920, xli, 327.

Masugi, M. Ueber die Beziehung zwischen Monocyten und Histiocyten. *Beitr. z. path. Anat. u. z. allg. Path.*, 1927, lxxvi, 396.

Maximow, A. A. Tuberculosis of mammalian tissue in vitro. *J. Infect. Dis.*, 1924, xxxiv, 549.

Maximow, A. A. Ueber undifferenzierte Blutzellen und mesenchymale Keimlager im erwachsenen Organismus. *Klin. Wchnschr.*, 1926, v, 2193.

Maximow, A. A., Development of non-granular leucocytes (lymphocytes and monocytes) into polyblasts (macrophages) and fibroblasts in vitro. *Proc. Soc. Exper. Biol. & Med.*, 1927, xxiv, 570.

Metschnikoff, E. Ueber die phagocytäre Rolle der Tuberkelriesenzellen. *Virchows Arch. f. path. Anat.*, 1888, cxiii, 63.

Metschnikoff, E. Immunity in infective diseases. Tr. from the French by F. G. Binnie. Cambridge, Cambridge University, 1907.

Metschnikoff, E. Die Lehre von den Phagocyten und deren experimentelle Grundlagen. Handbuch der pathogenen Mikroorganismen. Kolle, W. u. Wassermann, A. von. Jena, 1913. 2 Auflage, ii, 655.

Ogawa, C. Contributions to the histology of the respiratory spaces of the vertebrate lungs. *Am. J. Anat.*, 1920, xxvii, 333.

Oppenheimer, R. Experimentelle Beiträge zur Histogenese des miliaren Lebertuberkels. *Virchows Arch. f. path. Anat.*, 1920, cxciv, 254.

Pagel, W. Zur Frage der Abstammung der Exsudatzellen bei käsiger Pneumonie. *Beitr. z. Klin. d. Tuberk.*, 1925, lxi, 221.

Permar, H. H. An experimental study of the mononuclear phagocytes of the lung. *J. Med. Res.*, 1920, xlii, 9.

Permar, H. H. The development of the mononuclear phagocyte of the lung. *J. Med. Res.*, 1920, xlii, 147.

Permar, H. H. The pathogenesis of experimental pneumonia in the rabbit. *J. Med. Res.*, 1923, xliv, 1.

Permar, H. H. The mononuclear phagocytes in experimental pneumonia. *J. Med. Res.*, 1923, xliv, 27.

Permar, H. H. The function of the endothelial cell in pathological conditions, especially in tuberculosis. *Am. Rev. Tuberc.*, 1924, ix, 507.

Sabin, F. R. On the origin of the cells of the blood. *Physiol. Rev.*, 1922, ii, 38.

Sabin, F. R., Cunningham, R. S., and Doan, C. A. The separation of the phagocytic cells of the peritoneal exudates into two distinct types. *Proc. Soc. Exper. Biol. & Med.*, 1924, xxi, 330.

Sabin, F. R., Cunningham, R. S., and Doan, C. A. The discrimination of two types of phagocytic cells in the connective tissues by the supravital technique. *Contrib. Embryol.*, Carnegie Institution of Washington, 1925, xvi, no. 22, 125.

Sacks, B. The reticulo-endothelial system. *Physiol. Rev.*, 1926, vi, 504.

Sewell, W. T. The phagocytic properties of the alveolar cells of the lung. *J. Path. & Bact.*, 1918, xxii, 40.

Stewart, F. W. An histogenic study of the respiratory epithelium. *Anat. Record*, 1923, xxv, 181.

Stilwell, F. On the phagocytic capacity of the blood vessel endothelium of the frog's tongue and its presumed transformation into wandering cells. *Folia haemat.*, 1926, xxxiii, 81.

Timofejewsky, A. D., and Benewolenskaja, S. W. Zur Frage über die Reaktion von Gewebskulturen auf Tuberkuloseinfektion. *Virchows Arch. f. path. Anat.*, 1925, cclv, 613.

Töppich, G. Die celluläre Abwehrvorgänge in der Lunge bei Erst- und Wiederinfektion mit Tuberkelbazillen. *Krankheitsforschung*, 1925, ii, 315.

Watanabe, K. Versuche über die Wirkung der in die Trachea eingeführten Tuberkelbazillen auf die Lunge von Kaninchen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1902, xxxi, 367.

Westhues, H. Herkunft der Phagocyten in der Lunge. *Beitr. z. path. Anat. u. z. allg. Path.*, 1922, lxx, 223.

Westhues, H., and Westhues, M. Ueber die Herkunft der Phagozyten in der Lunge, zugleich ein Beitrag zur Frage der Funktion der Alveolarepithelien. *Beitr. z. path. Anat. u. z. allg. Path.*, 1925, lxxiv, 432.

Wislocki, G. Fate of carbon particles injected into the circulation, with special reference to lungs. *Am. J. Anat.*, 1924, xxxii, 423.

Wolbach, S. B. Studies on Rocky Mountain spotted fever. *J. Med. Res.*, 1919, xli, 1.

Wolbach, S. B., Todd, J. L., and Palfrey, F. W. The etiology and pathology of typhus. Am. Red Cross and Harvard University Press, Cambridge, Mass., 1922.

DESCRIPTION OF PLATE

PLATE 120

FIGS. 1, 2 and 3. These show cells supravitally stained with Nile blue sulphate. Fig. 1 shows the rosette form of granular arrangement and in Fig. 3 (young dust cell) a neutral fat globule has displaced the Nile blue sulphate granules.

FIG. 4. Monocyte stained with neutral red, supravital technic.

FIG. 5. Monocyte stained with Sudan III and hematoxylin, after fixation in formalin.

FIG. 6. Monocyte impregnated with silver tannate, after fixation.

FIG. 7. A typical dust cell, stained supravitally with Nile blue sulphate. It contains (*a*) Nile blue granules and vacuoles, (*b*) neutral fat droplets, (*c*) carbon particles and (*d*) refractile yellowish material. (Wislocki's "Type III Clasmatocyte".)

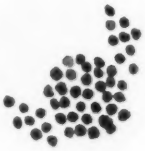
FIG. 8. Young dust cell, impregnated with silver tannate after fixation. Compare with Fig. 3. A fat droplet is present, but has not displaced the granules which form a rosette.

FIG. 9. A desquamated epithelial plate, supravitally stained with Nile blue sulphate. Note the Y-shaped structures and the general pallor of the cell, also the nuclear remnant.

FIG. 10. A bit of pulmonary capillary supravitally stained with Nile blue sulphate and drawn on a smaller scale. The vascular endothelium is free from granules.

The cells were outlined with a camera lucida and drawn in freehand.

Figs. 1 to 6 inclusive are semidiagrammatic drawings of monocytes stained in various ways.



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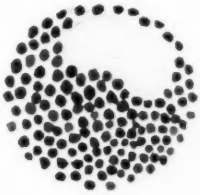

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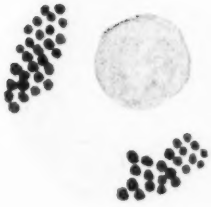

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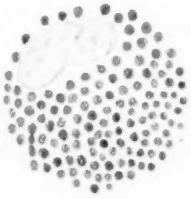

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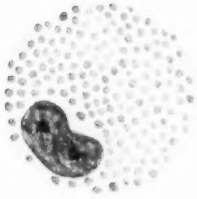

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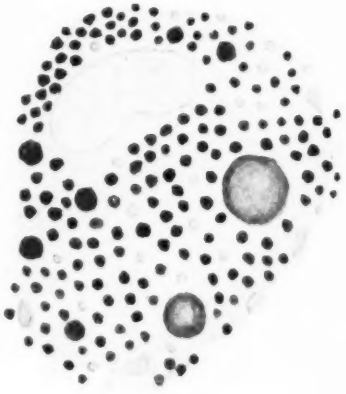

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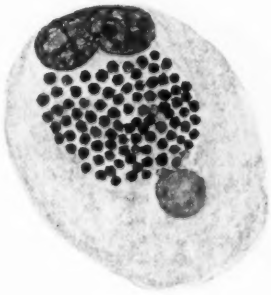

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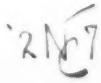

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Foot

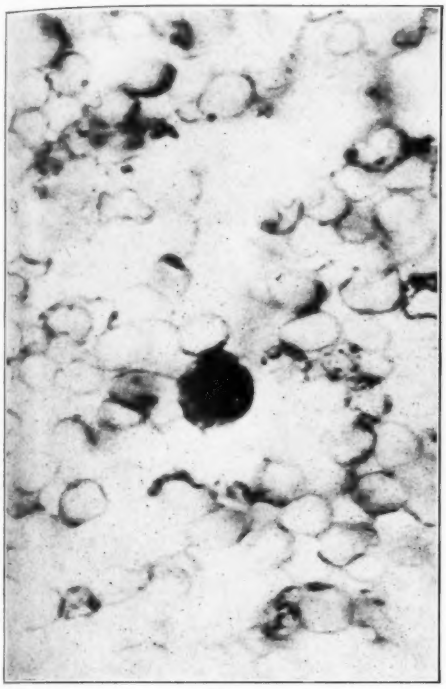
Origin of Pulmonary Dust Cell

PLATE 121

FIG. 11. A monocyte in the blood of a pulmonary vein. Note the granules and the "attraction sphere" in the bight of the nucleus. Silver tannate technic. Oil-immersion photomicrograph, about × 1000.

FIG. 12. A group of monocytes in a capillary knot in a rabbit's lung. One of them has either emerged from the capillary, or is lying in an alveolar "niche." (Fenestrum?) Note the granules in these cells and the total absence thereof in the capillary endothelium. Silver tannate, oil-immersion. × 1000.

FIG.. 13. Dust cells in the alveoli of a rabbit's lung. Note the grouping of the granules and the absence of these in the alveolar epithelium and capillary endothelium. Some of the cells show fat vacuoles. Silver tannate. × 500.



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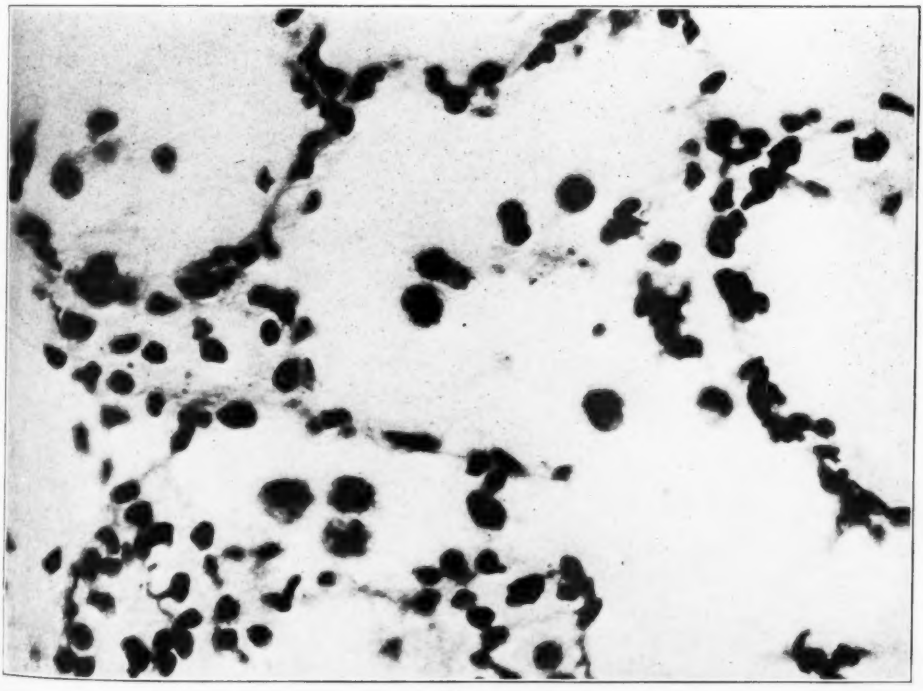

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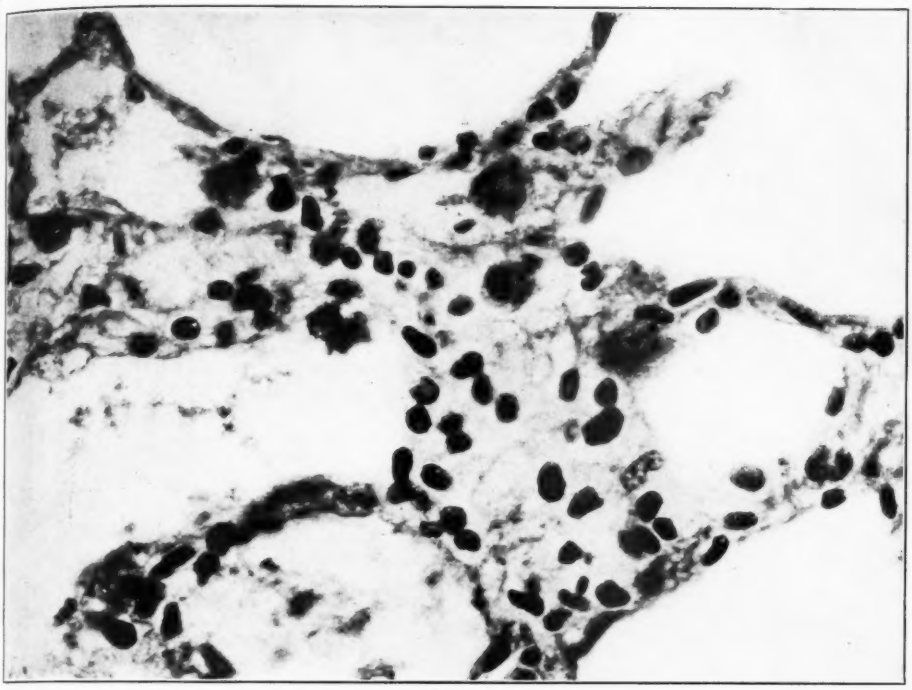
Foot

Origin of Pulmonary Dust Cell

PLATE 122

FIG. 14. Surface view of alveolar epithelium. Note the occasional fenestrations, the interposition of dust cells in these and the outlines of the epithelial "flanges" (non-nucleated plates). *Cf.* with Ogawa's drawings. ('20). There are no sizable granules in the epithelium. Silver tannate. × 500.

FIG. 15. A thickened area in a rabbit's lung 24 hours after the injection of milk. This illustrates the difficulty experienced in identifying the component cells in such foci of inflammation. Silver tannate. × 500.



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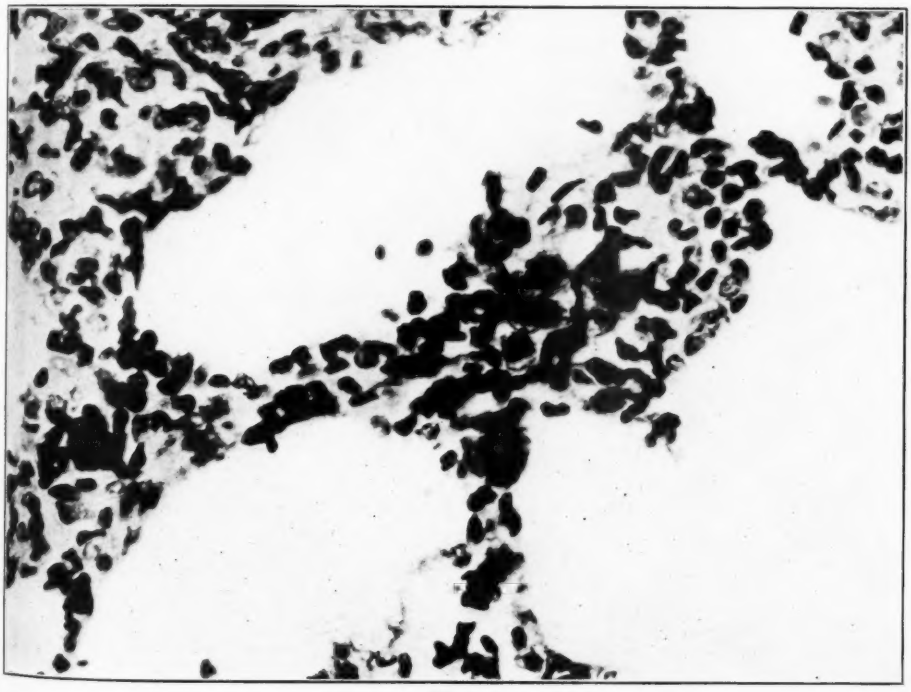

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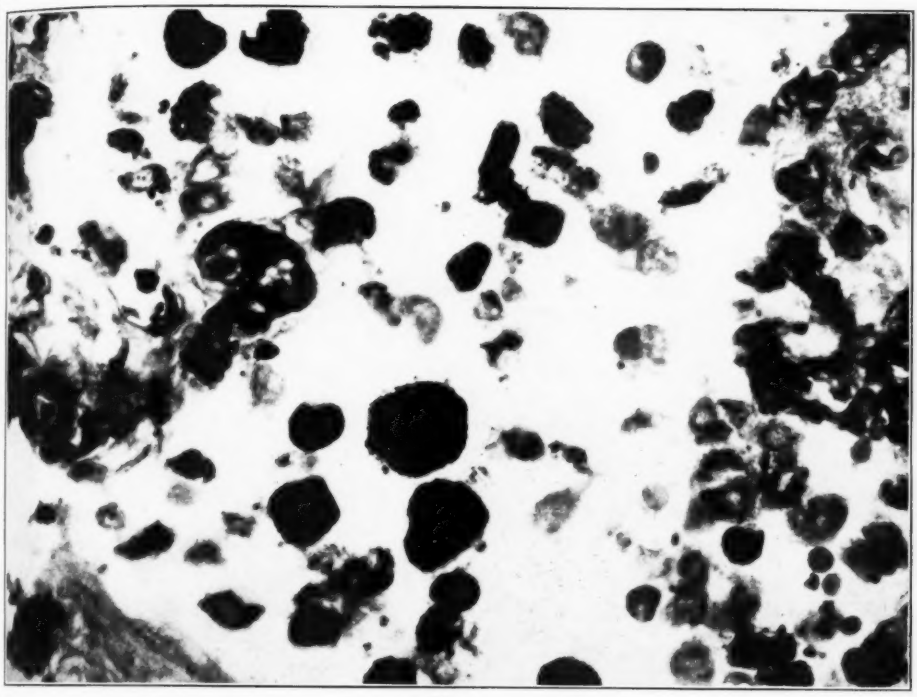
Foot

Origin of Pulmonary Dust Cell

Plate 123

Fig. 16. Alveoli in a human case of chronic passive pulmonary congestion. Note the dense granules in the "Herzfehlerzellen," the comparative pallor of the desquamated epithelial cells, and the monocytes near the alveolar walls. The small, free granules between epithelial cells are coagulated albumen in edema fluid. Silver tannate. × 500.

Fig. 17. Hematoxylin and eosin section from the same block as that in Fig. 16. Note the absence of all granules other than hemosiderin or carbon and the misleading similarity in the appearance of desquamated epithelium and the dust cells, or "Herzfehlerzellen." Compare with Fig. 16. × 500.



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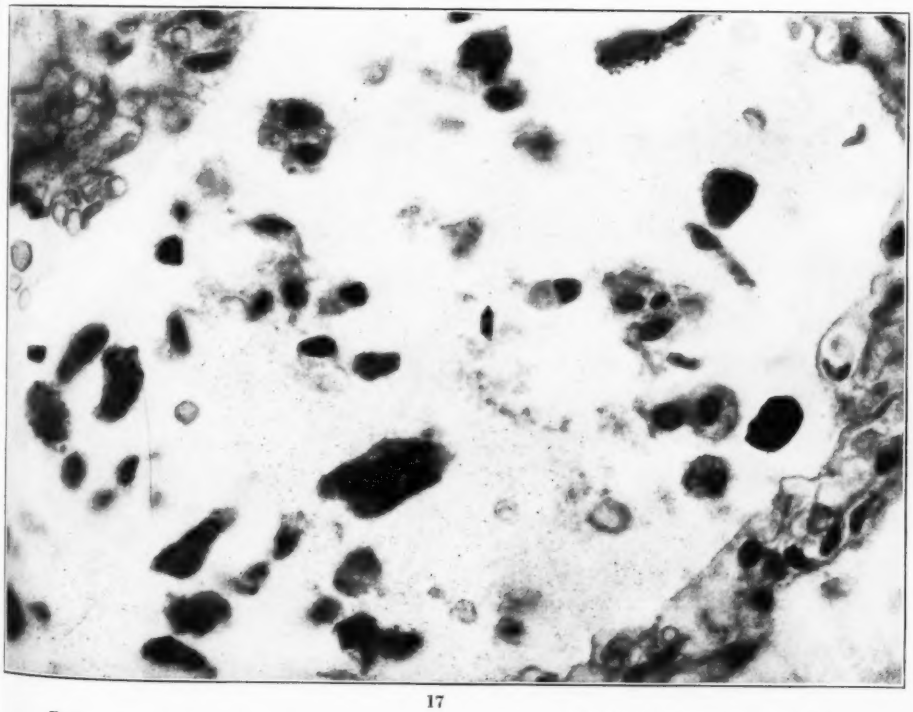

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Foot

Origin of Pulmonary Dust Cell

THE ORIGIN OF THE ALVEOLAR PHAGOCYTE STUDIED IN PARAFFIN SECTIONS OF TISSUE STAINED SUPRAVITALLY WITH NEUTRAL RED *

LEROY U. GARDNER AND DAVID T. SMITH

(From the Saranac Laboratory for the Study of Tuberculosis, Saranac Lake, N. Y. and from The New York State Hospital for Incipient Tuberculosis, Ray Brook, N. Y.)

So much has been written on the origin of the alveolar phagocyte that it would be useless to offer further discussion of the problem, unless approached from an entirely new angle. The perfection of a technic for preserving supravital staining of the lung in paraffin sections has furnished such a new means of attack. The use of supravital staining with neutral red, which has accomplished so much in the reclassification of blood and exudate cells, has hitherto been limited to smears and scrapings. Whereas such preparations demonstrate the types of cells present they give no information as to the topographical relationship of these cells. However, when it becomes possible to study in thin paraffin sections the cells stained supravitally as they appear in smears but preserved in their normal positions, a means is at hand to correlate the new cytologic data with the long established tissue morphology.

TECHNIC

The details of the technical procedures have been described elsewhere,[1] but it may be briefly stated that the cells of the pulmonary tissues in a living or freshly killed animal can be specifically stained by the intratracheal or intravenous injection of dilute solutions of neutral red in physiologic salt solution. Such staining can be preserved in sections embedded in paraffin by fixation in an alkaline Zenker-formaldehyde solution followed by rapid dehydration in mixtures of benzene and 95 per cent alcohol. The nuclei are counterstained with Harris' hematoxylin.

Both intratracheal and intravascular staining have been used because significant differences in effects have been observed. The former method stains all of the susceptible cells in the lung, those free in the air spaces and most of those in the blood and pulmonary tis-

* Received for publication June 29, 1927.

sues. Intravascular injections stain more especially the tissue and blood cells, whereas intra-alveolar cells often fail to assimilate the dye. The method which we have used is, in a sense, *supravital* in that the animal is killed by air embolism before injecting the dye. Staining is accomplished during a period of incubation of five to thirty minutes. While we were at work on the material to be reported, Cash [2] described *vital* staining with neutral red for the study of the histogenesis of the pulmonary tubercle. His method differs from ours in that he injected a smaller amount of concentrated dye into a vein ten minutes before killing the animal.

Our procedure has been as follows: In rabbits, 10 cc. of air is injected into an ear vein. In guinea pigs, which were most frequently used, the jugular veins are exposed under local anesthesia with novocain, and air is introduced. For intratracheal staining, the trachea is exposed in the neck, tied below the larynx and 30 to 60 cc. of a warm 1:1500* solution of neutral red in physiologic saline is injected into the lung. The whole animal is then incubated at 37° C. for a period varying from 5 to 30 minutes. The lungs are quickly removed and dropped into the fixative.

For intravascular staining, in most instances 50 to 80 cc. of the same dilute solution of dye is injected into a jugular vein; in some cases it has been injected into the carotid artery or the right ventricle of the heart. The dye may be introduced without first killing the animal by air embolism, but a guinea pig usually dies after the injection of more than 20 cc.

Description of Lungs Stained by Neutral Red

Neutral red injected intravenously stains chiefly the cells of the normal lung structure; introduced by way of the trachea it not only stains the pulmonary cells, but it is sufficiently irritating, even in dilute solutions, to provoke the formation of a specifically stained mononuclear exudate in the air spaces. Under these conditions the whole process occurs after the death of the animal when the circulation has ceased. In some respects this stimulating capacity of dye is detrimental but we have turned it to advantage for the staining

* In our original description of the technic [1] we prescribed a dilution of 1:1500. With Grübler dye this is satisfactory. Subsequently we have used the product of The National Aniline Company and find that their more concentrated dye should be diluted 1: 2000.

solution has served both as excitant and stain for the cells under investigation.

The reaction of the different types of cells to neutral red approximates that seen in smears and scrapings. However, fixation tends to neutralize the more delicate gradations of shade seen particularly in the clasmatocyte or macrophage. Furthermore it must be remembered that in sections the cells are cut at various angles, so that all planes cannot be brought into view as they can in smears. With these exceptions the cells in sections closely resemble those in smears.

A. Intravascular Injections. When the stain is introduced into the vascular system it acts only as a dye and does not exert any appreciably irritating effect. When viewed with low power a section of a normal guinea pig's lung exhibits a surprisingly large number of brilliant, red granular cells situated in the connective tissues of the trunks and alveolar walls. The characteristics of the various supravitally stained cells will be considered individually.

Epithelium. The columnar epithelium of the trachea and bronchi shows many large, deeply stained solid granules scattered at random throughout the cytoplasm. (Fig. 5.) The alveolar epithelium is best studied in thick sections (75 to 100 microns). These cells contain very few fine scattered cytoplasmic granules. (Fig. 1*a*.) In the ordinary thin sections (6 microns) these granules are occasionally found in the cut edges of the epithelium along the margins of the septa. The atypical vacuolated epithelium, which regenerates in chronic endemic pneumonia so common in guinea pigs, is apparently unable to retain the dye. In general, the arrangement of the stained cytoplasmic bodies of epithelium exhibits no characteristic pattern.

Endothelium. The cells lining the pulmonary blood and lymph vessels (Figs. 4*a*, 5*c*) do not stain specifically with neutral red, an observation which has been repeatedly verified in scrapings from the intima of larger vessels. In the specialized endothelia of the spleen, lymph nodes and liver (reticulo-endothelium and Kupffer cells) neutral red staining is very abundant but this does not concern us here.

Mesothelium. The cells covering the visceral pleura exhibit occasional fine cytoplasmic stained granules.

Clasmatocytes. (Histiocytes or macrophages.) Two types of this cell are found in the lung. The first regularly occurs in the connective tissues about the larger bronchi and vessels, in the intrapul-

monary lymphoid tissues and to a much lesser extent in the alveolar septa. This is the typical clasmatocyte, a large cell with a reniform or irregular nucleus surrounded by a cytoplasm which is packed with countless stained granules of variable size and shape (Fig. 1 *d*). Sometimes nuclear fragments of phagocytosed cells can be distinguished among them. The pattern of these granules is not peculiar. In color they vary from light orange to deep maroon. The number of such cells in the lung can only be appreciated in vitally stained tissues, for they are overlooked in ordinary hematoxylin and eosin preparations.

A second type of cell which we have also classified as a clasmatocyte is somewhat more conspicuous in sections stained in the usual manner. It is a large polygonal cell with vacuolated cytoplasm which projects from the surface of the alveolar septa. Sewell [3] described it as being particularly abundant in the corners formed by contiguous septa. He suggested that it might be a specialized type of epithelial cell, a view which is held today by Aschoff and his pupils.[4] Lang [5] named it the "septal cell" and identified it with the histiocyte. We shall employ his nomenclature for purposes of discussion. All of these observers, irrespective of their views as to its origin, affirm that this cell is directly or indirectly the source of the alveolar phagocyte.

Sections of lungs stained supravitally with neutral red bring out the great frequency of this cell in the septa. (Fig. 1*b*.) It projects into the lumen of the air space above and between the sparsely stained epithelial cells. Sometimes it assumes an hour-glass form which extends through the wall, the dilated terminal portions projecting into adjacent alveoli. (Fig. 2.) Neutral red is deposited in masses which occupy a large part of the cytoplasm. In size and color these masses are more uniform than in the typical clasmatocyte, but a certain degree of variation exists. In lightly stained preparations the deposits assume the form of brilliantly refractile orange-red signet rings of variable size. (Fig. 1*b*.) If the staining time is increased their annular form is obscured and the deposits become solid masses, varying from orange to red. Phagocytosed nuclear fragments have never been observed in this form.*

* Because of the resemblance of the ring-shaped deposits of dye to small erythrocytes it has been suggested that they represent phagocytosed fragments of these cells, but in appropriate control preparations the rings fail to stain with eosin or to give a microchemical reaction for iron.

The septal cell is peculiarly susceptible to overstaining with neutral red. With excessive exposures the stainable substance appears to be dissolved so that only its outlines are left, producing the vacuolization regularly seen in sections prepared by ordinary technics.

Tissue Eosinophiles. In the guinea pig's lung the connective tissues contain numbers of deeply stained, finely granular cells, often with elongated cytoplasmic processes. On closer examination it is seen that these granules are uniformly small and of a deep brick-red color. They stain more readily and resist decolorization more tenaciously than do the granules in any other type of cell. Their nuclei are bilobate or polymorphous.

By one accustomed to studying preparations of blood and exudates supravitally stained with neutral red, these cells would unquestionably be classified as mast cells because of the color of their granules. However, control sections of the same material, stained with hematoxylin and eosin or with eosin and methylene blue, exhibit correspondingly large numbers of typical eosinophiles in the same locations. Furthermore in lungs of rabbits, an animal notably deficient in true eosinophiles, this deeply stained, finely granular cell is extremely rare. For these reasons we are compelled to classify this cell as an eosinophile.

Blood Cells. Both within the vessels and after migration into the tissues the cells of hematogenous origin exhibit typical staining. The monocyte is characterized by the familiar rosette of fine granules situated in the "hof" of the nucleus. Lymphocytes show a few scattered neutral red granules if the plane of section happens to include them. Often, however, this is not the case and none is seen. The Kurloff bodies, which we believe occur in lymphocytes, are deeply stained. Polymorphonuclear neutrophiles are usually lighter and more yellow in color than those seen in smear preparations.

Fibroblasts. With prolonged exposure to the dye most of the fibroblasts contain occasional scattered pale orange to pink granules. Otherwise their appearance is not characteristic.

B. Intratracheal Injections. When introduced through the trachea, dilute solutions of neutral red not only stain the cells in the fixed tissues and blood stream, but provoke the migration of many beautifully stained phagocytes into the air spaces. In general the staining of the normal cells in the lung is the same as that seen after

intravascular injections of the dye. Only the effects upon the septal cells and the free phagocytes need further discussion.

Septal Cells. After an incubation period of five minutes following intratracheal staining the septal cells in no way differ from those stained intravenously. But thereafter many of them begin to lose their capacity to react supravitally. After ten minutes' exposure the previously annular deposits become solid and are much reduced in number. The cell now shows only a few large scattered granules in place of the closely packed ringlets which formerly filled the entire cytoplasmic substance. After thirty minutes there are practically no granules and the nucleus is tinged with red. One hour after staining the cytoplasm is filled with colorless vacuoles and even the nucleus has lost all traces of neutral red.

A much more significant reaction to intratracheal injections of neutral red is a marked diminution in the number of septal cells after prolonged irritation. Many sections from animals incubated from thirty minutes to one hour or longer show septum after septum without a single one of these cells. The diminution in the number of cells is not merely an apparent one because of their failure to stain, for the degenerative changes just described are always sufficiently definite to mark them if they are present. We believe that a desquamation of septal cells has occurred provoked by the presence of an irritating solution in the air spaces.

Free Alveolar Phagocytes. As early as five minutes after intratracheal injection of a dilute solution of neutral red the air spaces contain many large cells, each of which is characterized by the presence of a compact rosette of brilliant orange granules situated in the "hof" of an indented nucleus. The cells and the components of the rosette are much larger than those of the typical monocyte. In ten minutes the number of these free cells has greatly increased, and there are some with two or three nuclei. By thirty minutes they have become still more numerous and giant cells with as many as twelve or fourteen nuclei can be found. After an incubation period of one hour most of the dye has disappeared. A few of the free alveolar cells still retain large scattered granules and very rarely the remains of a rosette can be detected. Occasionally typical monocytes are encountered but these are rare except in the earliest periods. Polymorphonuclear leucocytes and lymphocytes are practically absent.

The rosette cell which suddenly appears in the air spaces does not resemble a monocyte. It is nearly twice the size of that cell and, with the lapse of time after staining, the dye granules constituting its rosette begin to exhibit variation in shape, size and color. At five minutes they are ring-shaped like those of the septal cells, but later they become solid masses of color which varies from pale orange to deep maroon. The rosette formation is preserved for at least thirty minutes, but at that time a few granules of dye appear at the opposite side of the nucleus. Still later the dye appears to have been dissolved and excreted; the majority of the cells then stain only with hematoxylin. They are much more resistant to the toxic action of the neutral red than the septal cells. Nuclear staining is rarely seen.

During the period of thirty minutes after their appearance, the large rosette cells only occasionally become phagocytic. Even in the presence of a great abundance of available material they usually fail to assume this function. In the earlier stages of the work the animals were killed by section of the cervical vessels, and much blood was aspirated, yet these large rosette cells showed no ingested red corpuscles. Rarely, however, one does contain a carbon particle or two.

The multinucleated cells seem to be formed by fusion, but purely morphologic evidence is deceptive. They exhibit a peripheral ring of nuclei surrounding a very large central rosette. In some of the older preparations this rosette resembles that described by Cunningham, Sabin, Sugiyama and Kindwall [6] as characteristic of the epithelioid cell, an increase in the amount of stainable substance with a great multiplication of very fine particles at the center of the rosette and a segregation of the larger ones at the periphery.

Intratracheally, neutral red stains the alveolar epithelium in the same manner as intravascular injections but it causes no desquamation of these cells. In thick preparations it is possible to find areas in which the plane of section includes sheets of delicate nucleated cells covering the entire surface of an alveolus. These cells retain their original position after prolonged irritation and do not migrate like the septal cells.

As the duration of the irritation becomes greater, increasing numbers of typical small monocytes are found in the connective tissues, particularly about the vessels and bronchi. A few can be found in

the alveolar septa. Monocytes are less numerous in these recently killed animals, than in those to be described later where the dye is injected into the lungs during life.

Supplementary Observations

So astonishing was the very rapid accumulation of large rosette cells within the air spaces in the short period of five minutes that it seemed necessary to be certain that none was present at the time when the dye was injected. Accordingly a series of one-day old guinea pigs, in whose lungs the absence of dust precludes the presence of many phagocytes (Tchistovitch [7]), were stained in the same manner. In their lungs also the same rapid accumulation of large rosette cells followed the injection of the dye.

To study the reaction of more mature alveolar phagocytes to neutral red, the lungs of animals exposed for a year or more to the inhalation of coal, carborundum, quartz and granite dusts were stained intratracheally. These lungs were known to contain great numbers of dust-filled phagocytes which had remained in the lung for a prolonged period. In their lungs the dye provoked a fresh accumulation of large rosette cells but the dust particles were contained in older cells exhibiting not a rosette arrangement but the diffusely scattered staining characteristic of a clasmatocyte. Only rarely a fragment of dust was found in a cell with the rosette grouping.

To ascertain whether the reaction in the animal recently killed was the same as that in the living guinea pig, the following experiment was performed: A group of four pigs were anesthetized by intraperitoneal injection of amytal. Holding them in an upright position 3 to 4 cc. of neutral red solution was allowed to drop into their nostrils. Subsequent necropsies showed that the major portion of the dye was aspirated into the lungs. They were killed by air embolus at intervals of thirty minutes to three hours afterwards. The right pulmonary artery was clamped, and about 30 cc. of dilute neutral red was injected into the right ventricle of the heart. This treatment restained the cells of the left lung, while those of the right lung exhibited only the effect of the primary intratracheal staining.

Study of the sections from the right lungs, stained only intratracheally during life, demonstrates the fact that within thirty minutes after instillation, the neutral red taken up by the majority of the cells is decolorized and reduced to a greenish black pigment.

Only the eosinophiles contain unchanged dye. The irritation in the living animal with an active circulation provokes an exudation not only of large mononuclear phagocytes, but of a few polymorphonuclear neutrophiles. The presence of stained eosinophiles, of exudation and of changed pigment is conclusive evidence that the dye successfully penetrated the lung of the living animal.

Sections of the opposite lungs, restained through the blood vessels, demonstrate the fact that the cells containing the decolorized dye do not lose their capacity to react. Free phagocytes containing the older greenish black particles of the intratracheal neutral red again take up the fresh stain which is deposited in diffusely scattered granules and vacuoles having a characteristic clasmatocyte grouping. In many instances the new dye is superimposed upon the old. Some of the cells contain phagocytosed cellular débris. The color of the stained elements varies from light orange-pink to deep maroon. Giant cells are rare but they do occur, particularly in the animal killed at thirty minutes.

The septal cells also exhibit double staining and many of them now react like clasmatocytes. Evidence of diminution in the number of these cells is hard to demonstrate in the living animal, but the impression is gained that shortly after irritation the septa surrounding air spaces which contain many free phagocytes show fewer septal cells than those in other areas. Three hours after intranasal treatment the septa seem to contain even more than the normal number of these cells.

Typical small monocytes appear in large numbers in the connective tissue at thirty minutes after intranasal staining and in the alveoli after three hours.

An elimination of injured clasmatocytes from the lung is suggested by the appearance of partially stained cells of this type in the lymphatics. Their number steadily increases from one to three hours after intrapulmonary irritation.

To ascertain whether the cells of the blood retained in the pulmonary vessels after the circulation has ceased, play a part in the formation of alveolar phagocytes, lungs perfused with Ringer's solution were stained intratracheally. Six guinea pigs were anesthetized with amytal. The abdominal aorta was cut, a clamp was placed upon the inferior vena cava and 50 cc. of warm Ringer's solution containing heparin, one part in 10,000 was injected through an in-

ternal jugular vein. The perfusion was continued about five minutes. Clear fluid returned through the incised aorta and the lungs were apparently bloodless. Neutral red was then introduced through the trachea in the usual manner and the bodies were incubated twenty to thirty minutes.

Sections of these lungs demonstrated an almost complete removal of blood cells from the vessels. Alveolar phagocytes are formed in the same abundance as seen in the non-perfused animal. However, these cells do not exhibit the large rosette which was so characteristic in the former group. The neutral red granules are scattered diffusely throughout the cytoplasm, an arrangement more suggestive of the clasmatocyte type. The cells appear much swollen, a manifestation of the generalized edema produced by perfusion. Monocytes are extremely rare but in the connective tissues occasional unstained cells are encountered whose general morphology is suggestive of this type.

To determine whether capillary endothelium may be so altered during intrapulmonary irritation that it acquires the property of reacting to neutral red, the following experiments were tried: Without anesthesia, 2 cc. of filtered India ink, diluted 1 to 4 with distilled water, were allowed to run into the nostrils of 10 guinea pigs. They were killed by air embolism at intervals from 30 minutes to 4 days. All but one animal showed gross pigmentation of the lungs with ink. The lungs were stained by the intratracheal injections of neutral red.

In no instance is it possible to demonstrate specific staining of vascular endothelium and no evidence of division of this cell can be detected. The alveoli contain tremendous numbers of phagocytes exhibiting the diffuse clasmatocyte grouping of neutral red and the septa are crowded with similar cells. In both locations the majority of the cells contain ink.

In six other animals an edema of the lung was produced by a two minute exposure to chlorine vapor as reported by Haythorn.[8] They died or were killed in thirty minutes to one hour. Subsequent intratracheal injection of neutral red demonstrated that the majority of the pulmonary cells were so injured by this treatment that they either completely failed to stain specifically or the staining was very weak. No significant changes in the vascular endothelium were observed.

Discussion

Alveolar phagocytes are not present in any great number in the normal lung, but they accumulate rapidly after intrapulmonary irritation. The rapidity with which they accumulate in the air spaces suggests that they arise from some source readily available. Three possibilities are conceivable: (1) Multiplication of preëxisting phagocytes; (2) migration of cells from the blood stream; (3) simple migration or multiplication followed by migration of some cell in the permanent structure of the lung. The first source, preexisting free cells, is eliminated because it was demonstrated that free phagocytes accumulate with the same rapidity in the air spaces of very young animals which, previous to irritation, contain no cells of this type. Even though there were preëxisting intra-alveolar cells, five minutes is too short a period to permit cell division of this magnitude to occur. The second source, the blood stream, is eliminated because alveolar phagocytes accumulate after the death of the animal, when the blood is no longer circulating. A possible origin from the blood cells retained in the pulmonary vessels has been eliminated in the experiment where the blood was thoroughly washed out of the vessels previous to intra-alveolar irritation and staining.

Even though the circulation was intact, this cell could probably be eliminated, for Smith, Dworski and Gardner [13] have reported a series of cell counts, made of blood from the right and left ventricles of the heart at intervals after inhalation infection with tubercle bacilli, in which the number of monocytes in the blood entering the lungs did not sufficiently exceed that in the effluent blood to account for the great number of epithelioid cells in the lung. We therefore eliminate monocytes from the blood as direct ancestors of the alveolar phagocyte. For these reasons discussion is limited to a consideration of some cell in the essential structure of the lung.

The local origins which have been proposed for the alveolar phagocyte are as follows: Alveolar epithelium, vascular endothelium and connective tissue clasmatocytes or histiocytes which include the septal cells of Lang. It becomes necessary to examine each of these cells and to discuss the claims made for them in the light of the evidence previously presented.

The staining of the general alveolar epithelium in no way re-

sembles that of the free phagocyte. It is difficult to believe that within five minutes time the sparsely scattered fine granulations of the epithelial cell could be transformed into the rosette of large densely stained rings or globules of dye seen in the free phagocyte. Furthermore the demonstration in the thick sections of an intact sheet of lining epithelium without evidence of desquamation is opposed to the view that the phagocyte is derived from this source. The contention of Aschoff and his pupils [5] that the septal cell is a special type of epithelium will be discussed later.

Vascular endothelium as a source of alveolar phagocytes has been extensively investigated by Haythorn [8, 9] Permar [10, 11] and Foot.[12] The evidence derived from irritation and supravital staining with neutral red offers no support to this origin. The vascular endothelium of the lung does not react specifically to this dye. The only evidence of staining has been in the nuclei of dying cells. The technic employed is adequate to demonstrate the presence of mitosis or indirect division. Prolonged search has failed to demonstrate a single example of proliferation in the local capillary endothelium. Even after preliminary irritation with India ink these cells have failed to exhibit evidence of multiplication or of specific staining. It must be admitted that we relinquish this origin with considerable reluctance for previously we ourselves were convinced that in general vascular endothelial cells were the source of such phagocytes. However, the data submitted prohibit this conclusion.

There remains only the clasmatocyte or histiocyte group of cells, in which we have included the septal cells of the alveolar walls. We agree with Lang [14] that septal cells are somewhat modified clasmatocytes, but our judgment is based on their reaction to neutral red in supravital staining. Both forms exhibit a cytoplasm filled with specifically stained granules which in light exposures assume the form of refractile signet rings, but as the staining time increases they become dense masses which vary in depth of color. The following differences exist. The clasmatocyte is regularly phagocytic if the proper material is available; the septal cell is only rarely so unless the alveoli contain excessive amounts of foreign substance. The nucleus of the clasmatocyte may be reniform or irregular while in the septal cell it is generally round. This variation in function and morphology might conceivably be due to differences in position.

On the other hand, Aschoff holds that the septal cell is modified

epithelium but in supravitally stained preparations it bears no resemblance to any other epithelial cell in the body, and much less to those in the alveoli and bronchi. The general lining cells of the alveoli show widely scattered very fine droplets of stain. Between these, along the connective tissue septa, the septal cell with its heavy masses of deeply stained granules stands out in sharp contrast. Furthermore, it has been shown that epithelial cells do not desquamate after irritation whereas septal cells do.

The supravital reactions of the septal cell to neutral red so closely resemble those of clasmatocytes in general that we have classified it in this group in spite of certain morphologic and physiologic differences. In this conclusion we have concurred with Lang [14] whose inferences were drawn from a study of tissue cultures.

The similarity between septal cells and free alveolar phagocytes is equally striking in supravitally stained tissues. Both present annular refractile globules after short exposures to the dye which later become dense and homogeneous and both show well defined variations in the color of their granules. On the other hand there are differences; the sessile cell exhibits diffusely scattered cytoplasmic granules while in the free cell, when it first appears in the air space, these granules are closely packed into a rosette situated in the "hof" of an indented nucleus. With age they become scattered and the rosette is lost. The stainable substance in the septal cell is destroyed or dissolved after prolonged exposure to the dye but the cell can recover so that the same elements will be restained. In the free cell the combination of the dye with the cytoplasmic elements is more resistant and is not destroyed so quickly. The free cell is phagocytic; the sessile cell usually is not.

We believe that under the stimulation of foreign substances introduced into the alveoli, the septal cells desquamate and are transformed into alveolar phagocytes. It is difficult to prove with certainty that the number of sessile cells is actually decreased because all of them are not shed at one time but the impression is strong that they are diminished five or ten minutes after intratracheal injection of neutral red. It is more obvious in the animal recently killed than in the living one, because in the latter regeneration commences at once and replacement of the desquamated cells is constantly taking place. In the living animal, three hours after intranasal instillation of the dye the number of the septal cells is very obviously increased,

a condition which has been interpreted as a manifestation of regeneration.

The source from which regeneration of septal cells proceeds cannot be absolutely proved. Mitotic figures or evidence of indirect cell division is wanting in any cell in the vicinity. However, it is noteworthy that within ten minutes after introducing the irritating dye into the air spaces large numbers of typical small monocytes, not previously encountered, become visible in the pulmonary connective tissues. Cunningham, Sabin and Doan [15] are convinced that such cells develop from indifferent reticulum cells in various parts of the body. Lacking any other origin it has occurred to us that these small monocytes may undergo a process of maturation and evolve as connective tissue phagocytes and septal cells. They appear on stimulation at the same time when the septal cells are desquamating and they again decrease in number and finally disappear after the climax of the reaction. A few leave the connective tissues and get into the air spaces, but the majority of them remain inside the framework of the lung.

Summary

The stages of the histogenesis of alveolar phagocytes as we have observed them may be summarized as follows: Under stimulation the septal cells are shed into the alveoli and are transformed into free phagocytes. New septal cells are formed from connective tissue monocytes which in turn arise from the indifferent reticulum cells.

In formulating this hypothesis we have not insisted that the neutral red stained masses must always retain the same pattern regardless of the position and physiologic state of the cell. The reticulum cell shows no staining, the tissue monocyte exhibits a small rosette in the "hof" of an indented nucleus, the clasmatocyte has diffusely scattered granules of various sizes and colors, while the free phagocyte displays different groupings which vary with its age, at first a rosette and later a diffuse distribution. This variation can be proved by observation in the last named cell, a fact which lends plausibility to the concept as a whole. Cytoplasm is labile and rearrangement in its structure must occur with changes in mechanical conditions and the assumption of new functions.

In arriving at this conclusion we believe that we have offered further evidence to substantiate the observations of Lewis [16] that

alveolar phagocytes arise from monocytes. In the living frog she was able to observe a direct transformation of the cell in the blood into the pulmonary phagocyte, and she has also observed corresponding morphologic changes in hanging-drop preparations of blood.[16] In recently killed guinea pigs where the circulation is no longer functioning we have seen evidence that extravascular monocytes, probably produced locally in the connective tissues, gave rise to clasmatocytes and septal cells which in turn migrated into the air spaces and were there able to assume the function of phagocytosis. Our interpretation of this evidence has failed to substantiate the view of Sabin and her co-workers that the monocyte and clasmatocyte, as differentiated by supravital staining with neutral red, are two separate and distinct types of cells. We believe that the grouping of neutral red-stained elements in a cell can vary with its position and physiologic state.

Conclusions

The data obtained from a study of the lungs of guinea pigs stained supravitally with neutral red, immediately after death by air embolism, have suggested the following deductions:

1. The alveolar phagocyte is produced by the desquamation of septal cells.

2. The septal cell belongs to the general group of connective tissue phagocytes or clasmatocytes.

3. These clasmatocytes appear to arise from previously inactive reticulum cells which pass through a monocyte-like phase in the process of maturation.

4. Alveolar epithelium and local vascular endothelium play no part in the origin of the alveolar phagocyte.

5. The arrangement of the neutral red stained cytoplasmic elements is not fixed and cannot therefore be used as a criterion to differentiate clasmatocytes from monocytes.

REFERENCES

1. Gardner, L. U. *Proc. Soc. Exper. Biol. & Med.*, 1927, xxiv, 646.
2. Cash, J. R. *Proc. Soc. Exper. Biol. & Med.*, 1926, xxiv, 193.
3. Sewell, W. T. *J. Path. & Bact.*, 1918, xxii, 40.
4. Seeman, G. *Ziegler's Beitr.*, 1925, lxxiv, 345.
5. Lang, F. J. *J. Infect. Dis.*, 1925, xxxvii, 430.

6. Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A. *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 231.
7. Tchistovitch, N. *Ann. de l'Inst. Pasteur*, 1889, iii, 337.
8. Haythorn, S. R. Reported before Amer. Assn. Path. & Bact., Rochester, 1927.
9. Haythorn, S. R. *J. Med. Res.*, 1914, xxix, 259.
10. Permar, H. H. *J. Med. Res.*, 1920, xlii, 9.
11. Permar, H. H. *J. Med. Res.*, 1920, xlii, 209.
12. Foot, N. C. *J. Exper. Med.*, 1923, xxxvii, 139.
13. Smith, D. T., Dworski, M., and Gardner, L. U. Trans. Nat. Tuberc. Assn., 1927, 257.
14. Lang, F. J. *Arch. f. exper. Zellforsch*, 1927, ii, 93.
15. Cunningham, R. S., Sabin, F. R., and Doan, C. A. Contributions to Embryology, No. 84, Carnegie Institution of Washington, 1925, xvi, 229.
16. Lewis, M. R. *Bull. Johns Hopkins Hosp.*, 1925, xxxvi, 361.
17. Lewis, M. R. *Am. J. Path.*, 1925, i, 91.

DESCRIPTION OF PLATE*

PLATE 124

FIG. 1. (*a*) Alveolar epithelium; (*b*) Septal Call; (*c*) Free Phagocyte, "large rosette type"; (*d*) Free phagocyte, older form with clasmatocyte grouping of neutral red; (*e*) Polymorphonuclear neutrophile, degenerated form; (*f*) Free phagoctye, monocyte-like form; (*g*) Eosinophile.

FIG. 2. Septal cell projecting into adjacent alveoli.

FIG. 3. Fibroblast.

FIG. 4. Giant alveolar phagocyte; (*a*) and (*a*) capillary endothelium.

FIG. 5. Bronchial epithelium; (*a*) Eosinophile with elongated processes; (*b*) Polymorphonuclear neutrophile; (*c*) Capillary endothelium.

FIG. 6. Polymorphonuclear neutrophile.

FIG. 7. Blood vessel. Endothelium and smooth muscle show no specific stain; (*a*) Polymorphonuclear neutrophile; (*b*) Eosinophile; (*c*) Monocyte.

FIG. 8. Mitotic figure in a cell applied to the surface of an alveolar septum.

* All drawings made from preparations of animals stained intratracheally.

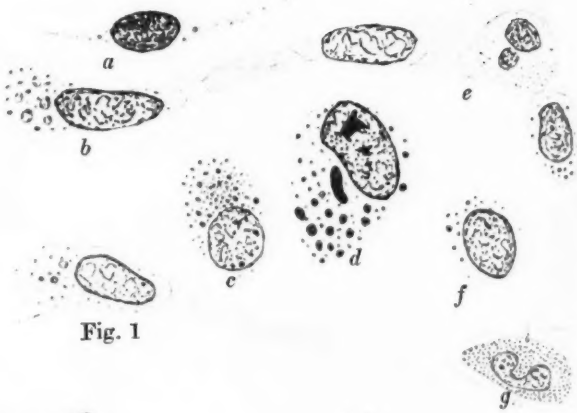


Fig. 1

Fig. 2

Fig. 4

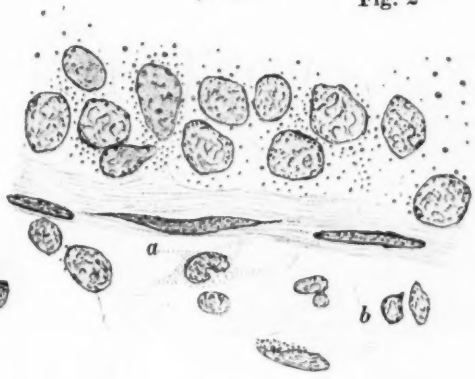


Fig. 5

Fig. 3

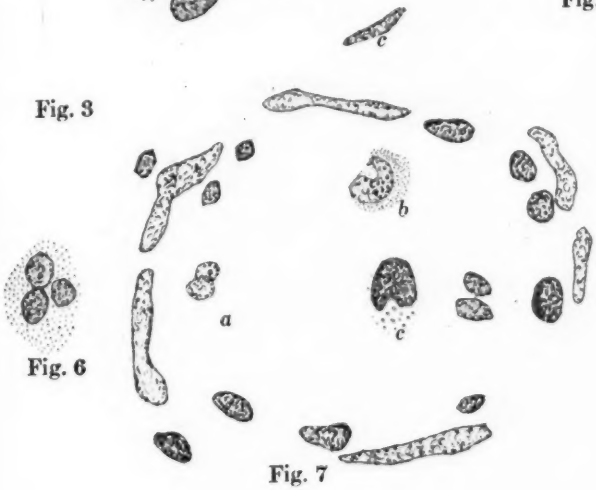


Fig. 6

Fig. 7

Fig. 8

HYALINE DEGENERATION OF THE ISLANDS OF LANGERHANS IN NON-DIABETICS *

ARTHUR WILLIAM WRIGHT, M.D.

(From the Department of Pathology, Vanderbilt University Medical School, Nashville, Tenn.)

Abundant evidence is at hand today to prove a causal relationship between degenerative or destructive lesions of the islands of Langerhans and the metabolic disturbance, diabetes mellitus. Initiated by the experiments of von Mering and Minkowski [1] in 1889, in which glycosuria was found to follow total extirpation of the pancreas in dogs, the relationship of the pancreas to this disease has been the subject of many investigations. In general these studies have followed one of two courses: first, the strictly experimental, in which a study of pancreatic physiology, both normal and abnormal, has been carried out through controlled animal experimentation; and second, the purely descriptive, in which the pancreas of the diabetic human has been made the subject of thorough morphologic study. In this second field little advance was made until about 1900 when Opie, in a series of papers, reported the results of his careful observations. Up to this time the pancreas was not thoroughly and systematically studied with the microscope, and most of the descriptive pancreatic pathology was based on macroscopic findings. It was Opie who described for the first time the most important histologic changes in the diabetic pancreas, and who called attention to the frequency with which the islands of Langerhans were involved. To one of the insular lesions, a degenerative process associated with the presence of a peculiar hyaline substance, described first in 1901, I wish to call special attention.

In his original paper, Opie [2] described a case in which the pancreas was the seat of a remarkable lesion which appeared to involve chiefly the islands of Langerhans. Throughout the organ there were distinct, sharply outlined, hyaline areas embedded in the parenchyma. These areas varied in size and were most numerous in the tail. Histologically, they consisted of thick columns of a homogeneous, hyaline substance which was present outside the capillary endothelium

* Received for publication May 31, 1927

and which separated the parenchymal cells from the neighboring vessels. The islet cells were atrophic or absent, and when the process had progressed, the whole structure was replaced by hyalin. In many places, so advanced was the destructive change, the hyaline substance was found to extend beyond the limits of the islands into the surrounding interacinar connective tissue. The primary lesion, however, was quite evidently within the insular spaces.

In a subsequent paper, Opie [3] reported another case in which hyalin was found, this time definitely confined to the Langerhans areas. The degree of involvement varied. Rare islets appeared to be uninjured. In others there were only a few small scattered masses of hyalin here and there in the island, closely associated with the capillary walls. Where the lesion was most advanced the island was practically replaced by the hyaline, homogeneous material in which a few elongated, compressed, pycnotic nuclei of parenchymal or endothelial cells remained. Often the parenchymal cells had entirely disappeared.

Following Opie's reports in which this peculiar hyaline degeneration of the islets was first described, there appeared other descriptions of a similar change, likewise limited to the insular tissue, in diabetic pancreases, Wright and Joslin,[4] Schmidt,[5] Herzog,[6] Müller,[7] Norris,[8] Cecil [9] and others. In every way the hyaline material was similar in nature and distribution to that described by Opie.

In all of these reported cases showing hyaline degeneration of the islets, diabetes mellitus was present. Not only was the disease itself evident, but it had usually existed for a long period, at least one year, and often three or more. The average duration in sixteen of the twenty-seven cases cited by Cecil was three years and six months. Furthermore, the lesion rarely occurs in patients under forty-five years of age. Of twenty-seven cases, Cecil found six who were under forty-five, while Warren and Root [10] report a single patient out of thirteen who was under forty-five. Cecil's youngest was a boy of twelve.

The lesion, then, in about seventy-five to eighty per cent of the cases is present in patients over forty-five; and this type of degeneration thus characterizes a certain type of diabetes which is most commonly found after middle life. It may be present with or without a coexistent interacinar sclerosis, but is rarely found associated with an acute process. The insular changes which are found in younger

individuals consist chiefly of inflammatory lesions, in which leucocytic infiltration of the islands is present in the early stages, and moderate to advanced sclerosis in the late. Rarely hyalin also may be found in these late inflammatory lesions. Occasionally in young patients the islands show no noteworthy morphologic alterations, as was noted by Cecil in nine of twenty-six patients under forty-five. The usual lesion, however, is infiltration of the insular bodies with leucocytes, or replacement of the islet parenchyma with scar tissue. The hyaline change, then, is characteristic of a group of cases in which the duration of the disease has been of some length, usually more than three years, and in which the patients themselves are middle aged or older.

In only one instance have I been able to find any reference to hyaline changes occurring in the pancreas of a patient in whom diabetes mellitus was not present clinically. Ohlmacher [11] described a case, clinically non-diabetic, in which there was hyaline degeneration of the islets. Cirrhosis of the liver was also present. In this case there was great hypertrophy of the unaffected islets, a finding which Ohlmacher thought might account for the absence of glycosuria. With the exception of this single case, then, hyaline degeneration of the islands of Langerhans has always been definitely associated with diabetes mellitus.

In this paper I wish to report five cases which came routinely to necropsy at the Vanderbilt University Hospital, and which show varying degrees of hyaline degeneration of many of the pancreatic islands. In none of these patients were there symptoms of diabetes mellitus during life, and in only one case was a trace of glucose found in the urine on one examination. The individuals came to the hospital for various reasons, and the pancreatic findings were incidental. All of the patients were men over fifty years of age, and they thus fall into the age group in which hyaline degeneration of the islets in diabetics is most commonly found. The clinical histories and necropsy findings are outlined briefly below.

CASE 1. *Clinical Report.* V–25–5. G. R., a negro, married, farmer, 53 years of age, was brought to the hospital Nov. 1, 1925, following an automobile accident. A diagnosis of fracture of the sixth cervical vertebra was made. Below that level there was almost complete paralysis and loss of sensation. Laminectomy was performed the morning after admission, but 36 hours later his temperature rose, respiration failed, and he died quietly.

There was nothing remarkable in the patient's past history.

The urine was examined once. It was amber in color, clear, and acid in reaction. Specific gravity 1025. Albumen and sugar were not present. Many pus and a few epithelial cells were found in the sediment.

A blood count showed moderate leucocytosis. Blood pressure, systolic 120, diastolic 80.

Necropsy Report. Necropsy begun one and a half hours postmortem. On gross examination the viscera are not remarkable. The pancreas shows no macroscopic changes. The main pathology is in the cervical region where there is separation of the fifth and sixth vertebrae with rupture of the posterior longitudinal ligament and severe crushing injury of the cord with hemorrhage into its substance.

Anatomic Diagnoses: Myelitis, transverse cervical (traumatic); multiple hemorrhages into muscles and soft tissues of the back; multiple subpleural hemorrhages; chronic pulmonary tuberculosis; focal necroses of the liver; hyalinization of the islands of Langerhans; old, healed peritonitis, localized; congenital abnormality, of the pelves of kidneys; hydrocele.

Microscopic examination of the pancreas: The tissue is well preserved. Casually examined, the sections reveal little which appears to be abnormal. The acini stand out clearly and are well stained. In occasional small areas there is slight increase in the interacinar connective tissue, but in general the interstitial tissue is normal. The larger arteries are moderately sclerosed, but the smaller vessels are normal. The islands of Langerhans are clear and distinct and are of fairly uniform size, averaging 100 to 250 microns in the largest diameter. In the majority of these structures there are no visible histologic changes, but here and there one finds small groups of islets which show the characteristic hyaline deposit. Of 300 islands counted at random in five sections from different parts of the organ, 96 show the hyaline change, so that approximately one-third are involved. The appearance of these altered islets is as follows: A varying amount of homogenous, hyaline substance which stains pink with eosin is present within the affected structure. Occasionally the hyaline masses, which are usually round or globular, appear to lie between groups of islet cells, but most commonly they are in intimate contact with the endothelial lining of the capillaries. Isolated masses of the substance sometimes lie at the periphery of the island, in contact with the fibrous capsule of the structure. The de-

gree of degeneration varies. In some of the islets there are only two or three small globules of the hyaline material, which appears to have little, if any, marked effect on the insular cells. In others, where the lesion is somewhat more advanced, the hyalin is present in considerable amount. It often appears to replace many of the cells which gradually atrophy or disappear. The cells which persist are compressed into small areas by the hyalin. They stain deeply and contain small, dark nuclei. Rarely one finds an islet in which the lesion is markedly advanced. Here the hyaline material is present in large amount in irregular rounded masses or globules which are conspicuous for their association with capillaries. Endothelial cells are still preserved and occasional red cells are seen within the narrowed lumina of the vessels. Between the hyaline masses, occasional compressed, elongated, or fusiform, darkly stained nuclei of parenchymal cells are found. In no case, however, does one find the entire islet replaced by the homogeneous material.

CASE 2. *Clinical Report.* V–25–6. L. B. M., a widower, 69 years of age, white, who worked as a clerk, was admitted to the surgical service of the Vanderbilt University Hospital, Sept. 27, 1925, complaining of hematuria, and pain in the lower abdomen. His past history was negative except for nocturia 2 to 12 times in the past 5 or 6 years. The present illness began 3 months before admission, with the appearance of blood in the urine. At first this symptom was unassociated with pain, but in the two or three weeks preceding entry he suffered a great deal. There had been a loss of 35 pounds in his weight during this recent illness.

Physically he was an obese individual with no other abnormal findings except those referable to the urinary system. A large tumor mass in the bladder was found on cystoscopic examination. On Oct. 13, 1925, the tumor was exposed, cauterized, and implanted with radium needles. The patient did well for about three weeks when deep X-ray therapy was instituted. To this treatment he reacted violently and died Nov. 6, 1925 with symptoms of alkalosis.

The urine on many examinations showed a trace of albumen but no sugar, except in a single specimen, taken just before he was removed to the operating room, in which a trace of glucose was found. In color the fluid was light amber or pale yellow, and clear. Specific gravity 1010 to 1021. On one examination there were many red cells. Later pus cells and organisms were found.

The leucocytic count was consistently high, ranging from 14,000 to 15,000 per c.mm. Blood non-protein nitrogen slightly elevated. Blood pressure, systolic 195, diastolic 75. Wassermann negative.

Necropsy Report: Necropsy performed forty-five minutes postmortem. A large tumor mass is found in the fundus of the bladder. It is necrotic throughout and its surface is ulcerated. This neoplasm definitely invades the perivesical tissues, and extends into the

wall of the right ureter which is occluded by the growth. Hydronephrosis is present on this side. A large pelvic abscess, apparently due to postoperative infection, is also exposed. The other anatomic diagnoses are abscess of the cortex of the left kidney; chronic pyelonephritis, left; chronic cholecystitis; occlusion of cystic duct by calculus; chronic vascular myocarditis; generalized arteriosclerosis. No gross abnormalities of the pancreas are noted, save for slight firmness or toughness.

Microscopic examination of the pancreas: Sections from the pancreas show a moderate ingrowth of fat tissue between the lobules of the organ, and groups of fat cells are not uncommon within the lobules themselves. The interacinar connective tissue is greatly increased in amount in all of the sections. In some of the lobules the increase is slight, appearing merely as small strands of fibrous tissue which run diffusely through the lobule. For the most part, however, the sclerosis is so marked that in many lobules the acinar tissue is greatly atrophied or has disappeared, leaving small numbers of islets isolated and embedded in dense scar tissue. Small focal collections of lymphocytes are frequently seen. The fibrosis is definitely of the interacinar variety; and about the larger ducts there is little, if any, increase in stroma. Arteriosclerosis of the pancreatic vessels is moderately advanced; there being frequently a diffuse thickening of the intimal lining of the arterioles.

In the lobules where the chronic inflammatory process is not marked, the islets show no noteworthy changes. They are sometimes considerably larger than normal; one, for example, measures 602 by 376 microns. In general, however, they average from 250 to 350 microns in the greatest diameter, which is within the normal limits. In the areas where interacinar sclerosis is prominent, however, many of the islets show distinct changes. In many, about twelve to fifteen per cent of the total pancreatic islands, there is an increase in the connective tissue stroma; that is, the islets themselves are the seat of moderate to advanced sclerosis. In several sections one may find occasional islands which, except for a few atrophic parenchymal cells compressed between strands of collagen fibrils, are entirely replaced by scar tissue. These, however, are relatively uncommon. In addition to this insular fibrosis, about ten per cent of all the islets show the typical hyaline deposit, with or without associated sclerosis. This lesion is less common than the

sclerosis alone. The hyaline masses are situated characteristically just outside the capillary endothelium, and only occasionally does one find an islet in which the change has progressed far enough to obliterate the structure almost entirely. In its miscroscopic appearances the hyaline material resembles that already described. In a few islets typical mitotic figures are found as evidence of regeneration of the parenchymal cells.

CASE 3. *Clinical Report.* V–25–13. J. A. C., 54 years of age, white, married, mill worker, was admitted to the Vanderbilt University Hospital, Dec. 14, 1925, in a dazed condition. He had been suffering with mental symptoms for about two years following an accident to his head. For six weeks prior to entry he had had spells of unconsciousness alternating with convulsions, apparently the result of a more recent accident in which he fell through a trap door and dropped several feet. Personality changes had been so noticeable of late that the patient was brought to the hospital.

As a result of thorough study the patient was thought to have either an abscess or a tumor of the brain. Since the Wassermann tests of both blood and spinal fluid were negative, and the colloidal mastic test not abnormal, syphilis was not considered. An operation was considered inadvisable and the patient grew gradually worse, dying Dec. 30, 1925.

The urine was not remarkable. It was amber, clear, and acid in reaction. No sugar was found. Albumen was not present. The sediment was negative.

The blood showed moderate anemia, 3,720,000 red blood cells per c.mm., and marked leucocytosis, from 14,000 to 24,000 per c.mm. 80 per cent of these were polymophonuclear leucocytes. Blood pressure, systolic 135, and diastolic 80.

Necropsy Report: Necropsy performed about six hours postmortem. On section of the brain a large, necrotic, edematous, cystic tumor (glioma) involving the basal ganglia and internal capsule on the left side is exposed. The remaining anatomic diagnoses are: Acute bronchopneumonia, terminal; central necrosis of the liver; parenchymatous degeneration of the kidneys; emphysema of the lungs; hypertrophy of the myocardium; adhesive pleuritis. The pancreas is not remarkable in gross.

Microscopic examination of the pancreas: Sections from the pancreas show moderate ingrowth of fat tissue between the lobules. There is no appreciable increase in the interacinar connective tissue, and evidence of an inflammatory process is lacking. Lobulation is distinct and the interlobular stroma is not increased. The tissue is well preserved except for occasional small foci of early postmortem degeneration. There is moderate arteriosclerosis of the smaller arteries. The islands stand out clearly in all sections. They are not enlarged nor do they appear to be increased in number. The ma-

jority of these structures show few, if any, noteworthy changes. Slight postmortem degeneration of some of the cells is sometimes seen, but otherwise these islets are not remarkable. Scattered about among these, however, are isolated islands or small groups of islands which show the characteristic hyaline change. In a total count of 300 of these bodies in several sections from different parts of the pancreas, ninety-four were found to show the hyaline change in some degree. Like those of Case 1, these islets contain varying quantities of hyalin. In general, the affected structures contain only small amounts of the substance and nowhere is the process advanced. There are no islands which are completely replaced by hyalin. The close relationship of the material to the endothelial lining of the capillaries is again observed. Several islets are found in which mitotic division of insular parenchymal cells is active.

CASE 4. *Clinical Record.* V–26–1. C. J. M., aged 57, married, white, locomotive engineer, was admitted to the surgical service of the Vanderbilt University Hospital, Dec. 5, 1925. The complaints were weakness, loss of weight, and swelling of the left chest; all symptoms of about a year's duration, with gradual increase in severity in the month preceding admission.

On physical examination the chest was found to be firm and flat to percussion, and breath sounds could not be heard. A diagnosis of carcinoma of the lung was made, and on December 8th, the patient left the hospital unimproved. At home he failed rapidly and died Jan. 2, 1926.

The urine was amber with a slight cloudy precipitate. It was neutral to litmus. Sugar and albumen were absent. A few pus cells were found in the sediment.

The blood examination was not remarkable except for moderate leucocytosis, about 17,400 per c.mm., of which 86 per cent were polymorphonuclear leucocytes. Blood Wassermann negative.

Necropsy Report and Anatomic Diagnoses: Necropsy performed two hours postmortem. Carcinoma of left lung with metastases to the pleura, oesophagus, liver, pancreas, adrenal, regional lymph nodes, and left femur; infarction of right lung; infarction of liver and spleen; focal necroses of liver; parenchymatous degeneration of kidneys; hypertrophy of prostate; thrombosis of left external iliac vein; arteriosclerosis, generalized.

Microscopic examination of the pancreas: Sections from various parts of the pancreas show tissues which are well preserved. The interlobular connective tissue is not increased in amount but in places it is edematous and infiltrated with large mononuclear leucocytes and lymphocytes. The walls of many of the arterioles are thickened and a dense, homogeneous, hyaline material is present in

the intima. In two sections, cords of atypical epithelial cells are found in some of the lymphatics, evidently a metastatic growth from the tumor of the left lung. The tumor, however, does not damage or destroy any of the pancreatic parenchyma. The pancreatic ducts are not remarkable except for occasional small, irregular concretions which are present in some of the lumina.

The interacinar connective tissue is not remarkable. It is not increased in amount and only in rare, inconspicuous foci does one find a slight chronic inflammatory reaction as indicated by the presence of small numbers of lymphocytes. The acini in general show no histologic abnormalities.

The islands of Langerhans are clear and distinct. The majority of these structures show no histologic changes but, as in Cases 1 and 3, varying degrees of the hyaline degeneration may be found in small numbers of them. In a count of 300 islets, sixty-two are found to contain some degree of the hyaline deposit, so that approximately twenty per cent of the structures are involved. The appearance of the hyalin varies in no way from that found in the other pancreases already described. In general, the affected islets contain only a small amount of the deposit, and no completely hyalinized islets are found. In size the islands are not remarkable. The largest structure in which hyalin is found measures 400 by 250 microns. The islets which are not involved vary somewhat in size, but only rarely does one encounter a structure which is beyond normal limits. The largest structure found measures 411 by 361 microns, and this is only slightly enlarged. A careful search for evidence of regeneration of the island cells fails to disclose mitotic figures.

CASE 5. *Clinical Report.* V–26–20. J. R., a negro, laborer, aged 55, was brought to the hospital, April 3, 1926, in a state of delirium. For several days previously he had felt sick, and on the day of admission he became suddenly unconscious while at work.

The physical examination showed a fairly well developed but rather poorly nourished adult negro in deep coma. Deviation of the eyes to the right, no reaction of pupils to light, a smooth right face, spasticity of upper extremities, tortuosity and thickening of peripheral vessels, and a blood pressure of 220 systolic, and 110 diastolic, led to a diagnosis of cerebral hemorrhage.

The patient was given morphine and a tap-water drip was started, but no improvement resulted. The coma grew progressively deeper and he failed rapidly, dying five hours after admission.

A single urine examination showed a considerable amount of albumen and a trace of sugar. A few granular casts and pus cells were found in the sediment. The fluid was straw-colored with a specific gravity of 1026.

Necropsy Report and Anatomic Diagnoses: Necropsy performed eighteen hours postmortem. Acute purulent meningitis, with considerable softening of the cerebral cortex; chronic vascular nephritis; passive congestion of the lungs; arteriosclerosis.

Microscopic examination of the pancreas: Sections from various parts of the pancreas show advanced postmortem degeneration which affects the entire organ. There is no increase in the interlobular connective tissue, but in places the interacinar stroma is considerably thickened and in these areas the acini are atrophied but the islets are not apparently affected. Foci of intimal thickening in the larger arterioles are seen. In general the insular bodies show little other than the changes associated with postmortem degeneration and a slight tendency to hypertrophy. Small numbers of the structures are definitely enlarged, two measuring, for example, 520 by 356 microns, and 465 by 210 microns, respectively. By far the larger number of islands, however, fall within the normal size limits. Here and there the typical hyaline deposit is noted. Out of 300 islets counted in several sections from various parts of the organ, hyaline material is found in only ten. About three to four per cent of the total islets are, therefore, involved. In no case is the hyalin present in large amount, not more than two to five small masses or globules being found in any one island. In its essential characteristics the hyaline material resembles that already described.

Discussion

Five cases are presented in which hyaline degeneration of the islands of Langerhans was found on histologic examination, but in which, during the lifetime of the individuals, there was no clinical evidence of diabetes mellitus. The patients came to the hospital for various reasons, three because of malignant disease, one as the result of an accident, and one with acute meningitis. Urine examinations in all cases but one were negative for glucose. This one patient (Case 5), showed a trace of sugar in the admission specimen, which was the only one obtained. In Case 2, a single test made just before the patient went to the operating room, revealed a trace of sugar, but many other examinations were consistently negative. Since, under conditions of undue nervous stress, glycosuria may be a physiologic phenomenon, little attention was paid to this isolated finding.

It is a noteworthy fact that all of the patients were over fifty years of age. This agrees with the findings of Cecil, Warren and Root, Opie,[12] and others, who, as has already been indicated, found in diabetics by far the greater percentage of hyaline degenerative changes of the pancreatic islands in adults over forty-five years of age.

These cases are taken from a series of eighty-four routine necropsies made at the Vanderbilt University Hospital during the period from Oct. 1, 1925, to Oct. 1, 1926. The pancreatic changes were found during microscopic study of the organs. In each case the pancreas revealed little on careful gross examination. In one of the cases (Case 2), which showed marked interacinar sclerosis, the organ was described as slightly firm and tough. The others were essentially negative, except for advanced postmortem changes in one (Case 5). The tissues were fixed in Zenker's fluid, several sections being taken routinely from the head, body, and tail of each pancreas, so that fair samples of the organs were available for microscopic examination. Five or six blocks of tissue, one or two from each part of the organ, were selected for embedding, and many sections were prepared.

Microscopic Examination. *Acinar tissue and stroma.* In three of the five cases no changes are observed in the acinar tissue. The glands are histologically normal. In the two remaining cases, however, where interacinar sclerosis is marked, there is atrophy and disappearance of much of the glandular tissue, accompanied by thickening and condensation of the surrounding stroma. In these cases there is further evidence of a chronic inflammatory process as shown by a mild infiltration of the connective tissue septa with cells of the lymphocytic series. In one case (Case 5), postmortem degeneration is so marked that a study of the acinar tissue cannot be made.

Blood Vessels. Arteriosclerosis is a common finding in all the patients, and intimal changes in the pancreatic arteries are moderately marked. In two cases there is considerable hyaline thickening of the intima of the smaller arteries, but this homogeneous material does not react to the various stains as does the hyaline substance which is found in the islets. The production of the insular hyalin is not dependent, as far as can be determined, on the arteriosclerotic process. The vascular changes appear to be merely coincidental and not in any sense causal.

Islands of Langerhans. In all five cases there is definite evidence of hyalinization of many of the islets. As studied in hematoxylin-eosin preparations, the hyaline material stains clearly pink and appears usually as a homogeneous, structureless substance. At times, however, the material seems to be arranged as fine, radiating fibrils which run from a central core and which give to the mass the appearance of a minute fuzzy ball. Occasionally, also, the material assumes a foamy appearance, due to the presence within it of numerous small vacuoles. These morphologic variations appear to be artifacts, since the greater part of the substance is amorphous, structureless, and smooth. Nearly always the hyaline masses are contained within the insular structures, only rarely being found outside.

The appearance of the islets which contain this material varies. Those which show the simplest type of involvement contain only one or two small masses of hyalin. These masses are present immediately outside of and adjacent to the endothelial lining of the local capillaries. The endothelial cells themselves are usually clear and distinct, no malformation being noted. The hyaline substance appears to rest within a small but definite space which is bordered on one side by endothelium, and on the other by islet cells. The islet cells are not abnormal except for slight pressure atrophy. The hyalin in most cases is entirely separate and distinct from the parenchymal cells, the borders of which are visible but often appear frayed and irregular.

Where the insular involvement is marked, the hyaline masses are more numerous, but they differ in no whit from those already described. About the capillaries the substance accumulates, forming a sort of cylinder which sheathes the vessel. In cross-section, then, the material is arranged in a circular fashion, in some places resembling a smooth, homogeneous ring; in others, where the substance is gathered into minute globules or spherical masses, appearing as a series of droplets encircling the vessel (Fig. 3). Where the capillaries are cut tangentially, the hyaline material assumes various shapes and forms, according to the plane of section. When the vessel is cut longitudinally, for example, the material is stretched out as irregular, wavy, homogeneous columns which run parallel with the blood channel (Fig. 1). It appears therefore that the hyalin collects chiefly about the capillaries of the tuft and, gradually accumulating, presses upon and causes atrophy of the parenchymal cells.

Only rarely is the hyalin present in such amounts that it replaces half or more of the islet (Fig. 1); and no completely hyalinized islands are found. Where the involvement is most marked, many of the parenchymal cells have atrophied, and some have disappeared. The cytoplasm of the atrophic cells stains deeply with eosin, and appears finely granular. The nuclei are small, pycnotic, and deeply stained with the basic dye. Chromatin granules are not usually visible, the nuclear stain being diffuse. The endothelial cells are flattened and compressed where the hyalin is present in moderately large quantity, and the capillary lumina are reduced in size. Rarely a completely collapsed capillary is found. In general, however, the hyaline degenerative process is not far enough advanced to produce marked capillary or parenchymal changes.

It seemed of interest to study more carefully the nature of this hyaline material, and sections from the various pancreases were subjected to a variety of staining methods. The material was uniformly negative when stained for amyloid with iodin-green or methyl violet, and amyloid was, therefore, readily ruled out. The reaction of the material to hematoxylin-eosin has already been mentioned. With eosin-methylene blue the substance stains deeply and homogeneously pink. By the use of Mallory's phosphotungstic acid hematoxylin the hyalin takes a light orange-brown stain, and stands out clearly from the surrounding purplish blue parenchymal cells. Stained by the aniline blue method as employed by Mallory for the demonstration of collagen, the hyalin takes a deep blue color, and forms a brilliant contrast from the bright red counterstain of the surrounding cells. The material, when stained with Van Gieson's picric acid and acid fuchsin mixture, takes the yellow color, rejecting the fuchsin. It thus behaves, according to Ernst, as does hyalin of epithelial origin. This homogeneous substance, therefore, stains exactly as did the hyalin described by Opie in the pancreases of diabetic individuals, and it may be reasonably assumed that we are dealing here with the same substance.

The hyaline material, according to Opie,[12] is of epithelial origin, the degenerative process first manifesting itself by changes in the protoplasm of the islet cells. Subsequently the cytoplasm becomes homogeneous, the nucleus disappears, and small particles of this altered, structureless cytoplasm fuse to form larger masses of hyalin which lie in contact with the fibrous septa of the islet. The cells in

which this degenerative process is taking place are stained a light blue color with Mallory's aniline blue, and resemble in a way the denser accumulations. Ultimately there is complete transformation of the parenchymatous cells, and the islet becomes a hyaline mass in which there remain only the altered capillaries.

In this study, many islands were carefully examined for the appearance of epithelial cell degeneration resembling that described by Opie. Here and there in some of the islets there appear to be occasional isolated cells which contain minute homogeneous masses which stain faintly blue by the aniline blue method of Mallory. These cell changes resemble very much the appearance described by Opie in his report of studies on sections stained by the same method. In general, however, these isolated, blue-stained, hyaline foci are clearly outside of epithelial cells, and appear to rest in small, definite intercellular spaces. Cell borders are often indented to form these openings, and the appearance is quite similar to that found in the liver in cases of chronic biliary stasis, where small globules of bile fill the distended intercellular ducts. These minute hyaline masses, when followed in serial sections, are commonly found to be portions of larger accumulations which are present either above or below the plane in which the isolated masses appear. There is thus but little evidence of a protoplasmic transformation within the insular cells, and the small isolated globules of hyaline material found in any single section are generally only portions of larger masses in another part of the islet.

The majority of the hyaline masses, however, are present about the capillaries, and they appear morphologically to be the result of a slow change which occurs in the fibrous tissue surrounding these vessels. The steps in the process are hard to follow. The first evidences appear to be the deposit or formation of a thin layer of homogeneous, hyaline material in the fibrous tissue adjacent to the endothelial lining of the capillary. This slowly accumulates and thickens up, gradually pushing the epithelial cells away. These cells undergo slow atrophic and degenerative changes, until, where the deposit is great, they ultimately disappear and are replaced by hyalin. This material, therefore, is formed outside the epithelial cells of the islets rather than from their cytoplasm. In spite of the reaction with picric acid and acid fuchsin, the substance seems to be a degenerative product formed as amyloid is, within the perivascular stroma.

The islets which are here involved show, therefore, slight to moderate hyaline degeneration. No structures are completely destroyed, and the pathologic process, morphologically, appears to be early. From islets in which two or three small droplets of hyalin are present, there are all degrees of involvement to those in which approximately one-half to three-quarters of the structure is replaced by the homogeneous material. These latter present the most advanced form of the lesion. In spite of atrophy and even disappearance of many of the islet cells, every injured islet studied contains some parenchymal cells which are not markedly abnormal.

In studying the pancreases of ninety cases of diabetes mellitus, Cecil found that twenty-seven showed the hyaline change. Twenty-one of these patients were 48 years of age or over, and thus fall into the age-group which most concerns us here. Of this latter group the degenerative lesion in the pancreases of seventeen was associated with varying degrees of chronic interacinar pancreatitis, while four showed no changes in the acinar parenchyma or stroma. In my five cases there are two which show marked interstitial sclerosis, but in only one of these does the scarring affect the islets themselves. In this case (2), insular fibrosis is more prominent than the hyaline accumulations, appearing in twelve to fifteen per cent of the structures, while hyalin is present in about eight to ten per cent. Rarely both lesions are observed in the same islet. In three cases, the hyaline change is relatively uncomplicated, only minute foci of interacinar fibrosis being occasionally found.

The relative number of islets involved in each of these cases is noteworthy, especially when considered from the standpoint of the relation of the hyaline degenerative process to diabetes mellitus. To get an approximate estimate of this involvement the following procedure was carried out. Three hundred islets, taken at random from the various sections available in each case, were counted and carefully studied. All structures showing even the slightest degree of hyaline deposit were noted and recorded. In Table I the relation of involved to uninvolved islets is indicated. One finds, as a result of this study, that in no case is more than one-third of the total number of islands affected by the degenerative process. In three cases considerably less than a third give evidence of the deposit. This fact again seems to point to an early stage in the process, since, in the majority of diabetic pancreases which show this type of change, it is

more common to find a larger proportion of affected than unaffected structures. Here, however, approximately two-thirds or more of the insular bodies are not morphologically abnormal.

Warren and Root, in studying the pancreases of diabetics who had been treated for a certain period of time with insulin, indicate that, in their opinion, regeneration of island cells can occur; and they cite cases of hemochromatosis and acute lobar pneumonia in which active regeneration is evidenced by the presence of one or more mitoses in single islets. In true uncomplicated cases of diabetes they found no evidence of regeneration. This they feel is due to the extreme chronicity of the course of the disease, where attempts at regeneration would be slow, and where mitotic figures would hardly be expected. Boyd and Robinson [13] also reported one case of diabetes in a boy who was accidentally killed after six months of insulin treatment, in whose pancreas there was definite evidence of regeneration.

TABLE 1

Summarizing the Findings of the Five cases Reported in this Paper

Case	Name	Age in years	Per cent of islands involved	Hypertrophy of uninvolved islands	Evidence of regeneration	Fibrosis of islands	Interacinar sclerosis	Arteriosclerosis
1	G.R.	53	32	None	None	None	Very slight	Slight
2	L.B.M.	69	8–10	Slight	Slight	12–15%	Marked	Moderate
3	J. A. C.	54	31	None	Slight	None	Very slight	Moderate
4	C.J.M.	57	20	Very slight	None	None	Very slight	Moderate
5	J.R.	55	3–4	Moderate	None	None	Moderate	Slight

It was of interest, therefore, to study the sections at hand from these five patients with a view to discovering whether or not insular regeneration was evident. In the majority of cases no mitotic figures within the islands could be found on careful high power study. In sections from the pancreases of Cases 2 and 3, however, several mitoses were discovered. These were evident not only in islets which were free from the hyaline deposit, but also in those in which

the degenerative change was relatively far advanced. The mitotic figures were definitely within the parenchymal cells of the islands, and could easily be distinguished from regenerative changes of the endothelium. The presence of this activity in the insular tissue shows the capacity of the islet cells to regenerate when injury has taken place, but does not, in either of these instances, prove that the regeneration is the direct result of a physiological deficiency in islet tissue alone. The necropsy findings in both of these cases showed evidence of acute infections. In Case 2 there was a large malignant tumor of the bladder with a secondary infection of the operative wound. This infection was acute, and blood stream invasion was indicated by infectious lesions in the kidney. Case 3 showed acute bronchopneumonia, with a moderate degree of toxic central necrosis of the liver. It may reasonably be assumed, therefore, that the injury in each case which resulted in regeneration was the acute toxemia rather than the slow, progressive insular change which was also present. Whether or not the considerable reduction in the amount of normal insular tissue available in these pancreases, has any effect on the readiness with which active regeneration will take place following a secondary acute toxic injury, cannot be definitely stated. It is not unreasonable to assume that, with a large number of islets seriously impaired by a hyaline or sclerotic change, the remaining normal tissue, if injured acutely, might regenerate more actively than the insular tissue in a pancreas which was otherwise normal. Of greatest importance here, however, is the fact that regeneration is evident in pancreases in which the characteristic hyaline degenerative process is relatively advanced, even to the point where new cell formation is observed in islets which themselves show the progressive, destructive change.

Ohlmacher, in reporting his case of hyalinization of the pancreatic islets without accompanying diabetes, calls attention to the large number of greatly hypertrophied islands. A careful study of the size of these structures was made in the present instance, and although occasional enlarged islands were found, the number which have undergone hypertrophy is not great. Further, some differences of opinion exist with regard to the size of normal islets. Koelliker,[14] reports the average size of the structures as from 70 to 300 microns. Laguesse,[15] on the other hand, states that normally many of the islets may measure as much as 400 microns in the greatest dimension.

Cecil, in his study of ninety cases of diabetes mellitus, considers 400 microns as the upper normal limit, and that dimension is used as a criterion here. Ohlmacher considered all structures measuring more than 300 microns in size as definitely enlarged.

Using a Zeiss ocular micrometer which had been previously standardized against a stage micrometer graduated in hundredths of a millimeter, the islands in all of the pancreases studied were accurately measured. In two of the cases (1 and 3), which show the largest percentage of hyalinized islets, no hypertrophied bodies are found. In the other three cases, however, small numbers of enlarged islands are present, the largest found measuring 602 by 376 microns (Case 2). In Case 4 the largest structure is but slightly beyond the normal limits, measuring 411 by 361 microns. In the last case a number of hypertrophied structures are present. In general the enlarged islets are free from the hyaline deposit. A careful search for mitoses within these bodies was unfruitful. One may conclude from these findings that hypertrophy of uninvolved islets does occur to a certain degree. Since, in these cases, a far smaller number of islands are affected by the hyaline degenerative process than were found by Ohlmacher in his case, the need for increased insular tissue is less marked.

It is evident that in the pancreases here described there is a slow, progressive, degenerative change which affects specifically the islands of Langerhans. Varying numbers of these structures contain a hyaline substance similar to that which other workers have described in the pancreases of diabetics. These other investigators have shown that the lesion is one which develops usually in later life and that only a limited number of cases of diabetes mellitus are ascribable to it. It is brought about gradually, and generally by conditions which are incident to the fifth and sixth decades, and its cause is entirely obscure. In my cases a relatively small number of the insular bodies are involved, and then only to a moderate degree. The change appears to be progressive, and if continued long enough would result in sufficient injury to the islets to cause a physiologic deficiency in the normal hormone, thus bringing about the clinical disease. It is believed that such is the course of events in cases of diabetes in which the islands, histologically, show the hyaline change. In the cases here reported the definite degenerative change is progressing in the islands, and in two cases definite evidence of active regeneration

of islet cells is demonstrated. In three of the cases hypertrophy of moderate numbers of uninvolved structures is also evident. These facts, together with the finding that less than one-third of the insular bodies are involved, and these to a moderate degree only, permit one to infer, I believe, that the degenerative process, whatever its nature may be, which causes the hyaline material to form in the insular structures, precedes the breakdown of the normal carbohydrate metabolic mechanism. These observations thus form another link in the chain of evidence which points to the part played by the islands of Langerhans in the utilization of sugar. Unquestionably in cases of late diabetes, where hyaline islets are found in great number, there must have existed a prediabetic period, in which slow changes in the islands, similar to those here described, took place. During this time some injurious agent, probably toxic in character, as suggested by Warren and Root, causes the slow formation or deposit of hyalin in the island. As the hyalin increases in amount it advances upon the normal parenchyma, causing atrophy and ultimate disappearance of the island cells. Since the process is very slow, the cells may regenerate, as has been demonstrated here. With the continued progress of the injury, however, the regenerated cells, as well as those which previously existed, are finally destroyed. With the advance of this process, there is slow and gradual destruction of more and more insular tissue until a point is reached where a sufficient amount of active islet parenchyma to produce the glycolytic hormone is not available. There then appear the phenomena of hyperglycemia and glycosuria. Concerning the regeneration of totally new islets, as suggested by Warren and Root, nothing can be said from the present study. That active regeneration may take place within the islands themselves is unquestionable, but whether or not new islands arise cannot be stated.

Summary

1. There are here reported five cases, clinically non-diabetic, in which the islands of Langerhans show the hyaline degenerative change characteristic of certain cases of diabetes mellitus.

2. All of these patients were over fifty years of age and thus fall into the age-group in which hyaline degeneration of the islets is commonly found in true diabetics.

3. The character of the lesions suggests a toxic origin, the injurious agent acting slowly and progressively over a very long period of time.

4. The degenerative process in each case affects less than one-third of the islets studied.

5. Tinctorially and chemically the hyaline substance resembles that described by Opie and others in cases of diabetes mellitus studied by them.

6. The material appears to be laid down outside the blood vessel endothelium, in the subjacent connective tissue, and is not a product of epithelial cell degeneration.

7. In two cases there is evidence of regeneration of the islet cells, not only of unaffected but of affected islets.

8. Moderate hypertrophy of some of the uninvolved islets is noted in three cases, but this bears no constant relation to the amount of insular tissue involved in the degenerative process.

9. One case shows moderate sclerosis of the islets in addition to the hyaline change.

10. Only two cases show a noteworthy increase in the interacinar connective tissue.

Conclusions

1. Hyaline degeneration of the islands of Langerhans is a lesion which develops commonly in later life and only a small number of cases of diabetes mellitus are ascribable to it.

2. It appears to be the result of slow toxic injury in which the hyaline material gradually accumulates, injures and finally destroys the islet cells, ultimately replacing the insular tissue. Regeneration takes place, but the regenerated tissue, under the influence of the continued action of the injurious agent, is again slowly destroyed. Gradually the regenerative power of the pancreas is worn down, and finally, when so much islet tissue is destroyed that the normal glycolytic hormone can no longer be supplied in sufficient amount, the phenomena of diabetes mellitus appear.

3. These cases may be classed as falling into a prediabetic group, sufficient insular tissue not being involved to bring about the clinical symptoms of diabetes mellitus.

REFERENCES

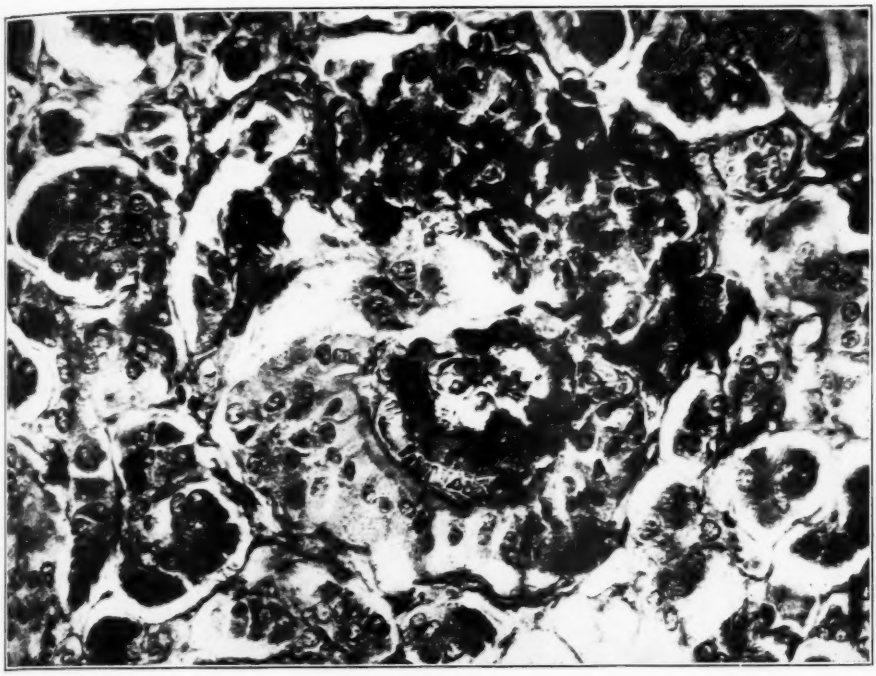
1. von Mering, J., and Minkowski, O. *Centralbl. f. klin. Med.*, 1889, x, 393.
   von Mering, J., and Minkowski, O. *Arch. f. Exper. Path. u. Pharmakol.*, 1890, xxvi, 371.
2. Opie, E. L. *J. Exper. Med.*, 1901, v, 397.
3. Opie, E. L. *J. Exper. Med.*, 1901, v, 527.
4. Wright, J. H., and Joslin, E. P. *J. Med. Res.*, 1901, vi, 360.
5. Schmidt, M. B. *München. med. Wchnschr.*, 1902, xlix, 51.
6. Herzog, M. *Virchows Arch. f. path. Anat.*, 1902, clxviii, 83.
7. Müller. Inaug. Diss., Berlin, 1905. (Cited by Opie.)
8. Norris, C. *Proc. New York Path. Soc.*, 1907, vii, 19.
9. Cecil, R. L. *J. Exper. Med.*, 1909, xi, 266.
10. Warren, S., and Root, H. F. *Am. J. Path.*, 1925, i, 415.
11. Ohlmacher, J. C. *Am. J. M. Sc.*, 1904, cxxviii, 287.
12. Opie, E. L. Disease of the Pancreas, Philadelphia, 1910. Ed. ii, 311.
13. Boyd, G. L., and Robinson, W. L. *Am. J. Path.*, 1925, i, 135.
14. Koelliker, A. Handbuch der Gewebelehre des Menschen, sechste Auflage, dritter Band, 1899, 253.
15. Laguesse, E. *Compt. rend. Soc. de biol.*, 1893, ix, 402;
    Laguesse, E. *Compt. rend. Soc. de biol.*, 1894, x, 667.

DESCRIPTION OF PLATE

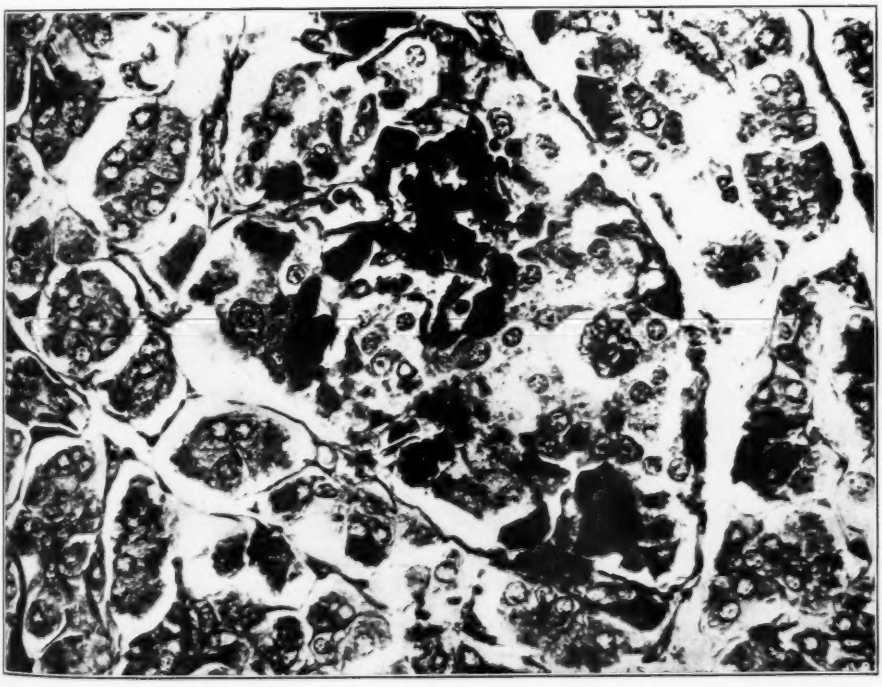
PLATE 125

FIG. 1. Case 3. Mallory's aniline blue stain. Small islet showing hyaline masses grouped about capillaries. One vessel, cut longitudinally, shows the wavy, hyaline columns running parallel with the wall. ×500.

FIG. 2. Case 1. Mallory's aniline blue stain. Islet showing several hyaline masses. ×500.



1



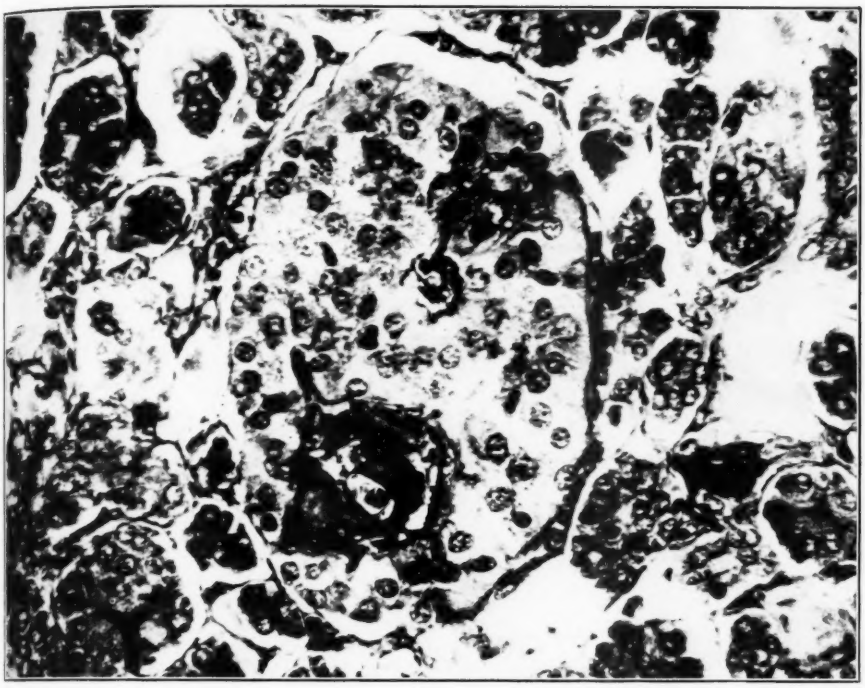
2

Wright Islands of Langerhans in Non-Diabetics

PLATE 126

FIG. 3. Case 3. Mallory's aniline blue stain. Island of Langerhans showing a thick collar of hyalin encircling a small capillary. ×500.

FIG. 4. Case 4. Mallory's aniline blue stain. Small island with multiple hyaline masses. ×500.



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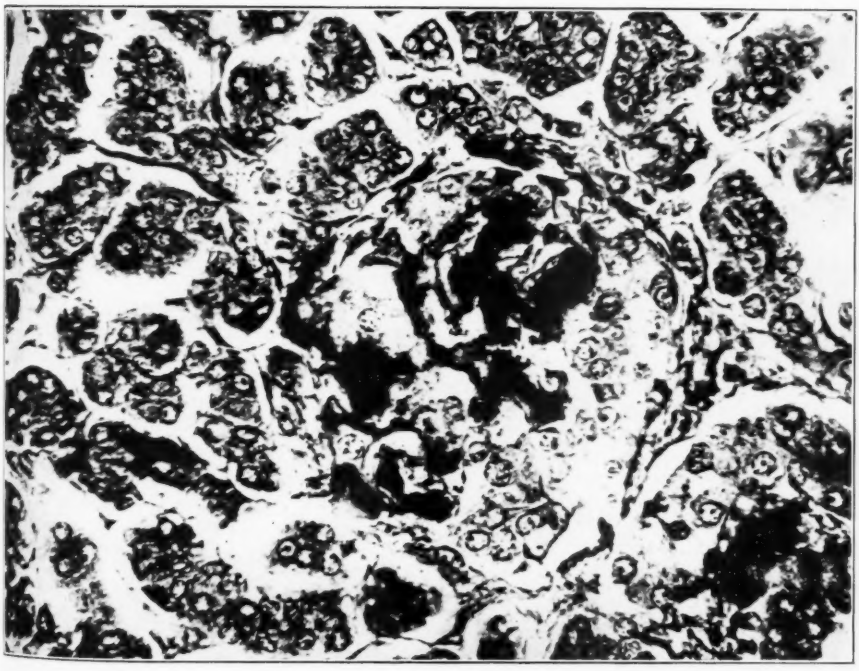


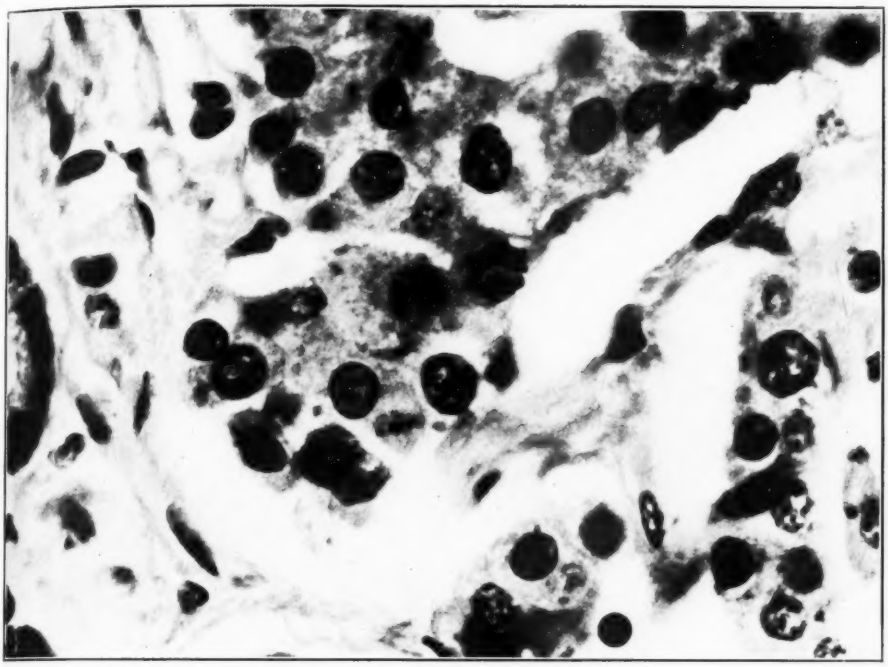
4

Wright Islands of Langerhans in Non-Diabetics

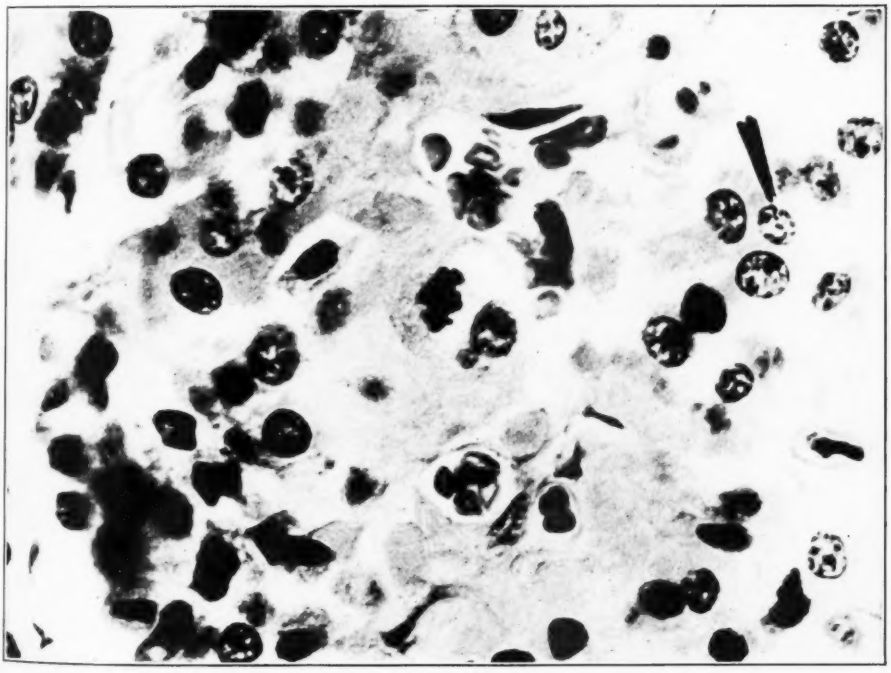
PLATE 127

FIG. 5. Case 2. Hematoxylin-eosin stain. One mitotic figure in an islet which contains none of the hyaline deposit. ×1000.

FIG. 6. Case 3. Hematoxylin-eosin stain. Mitotic figure in parenchymal cell of an island containing multiple hyaline masses. ×1000.



5



6

Wright Islands of Langerhans in Non-Diabetics

THE SPLEEN IN WEST AFRICAN YELLOW FEVER *

OSKAR KLOTZ AND WINIFRED SIMPSON

(From the Laboratories of Pathology, University of Toronto, Toronto, Canada)

Although the literature on yellow fever is fairly extensive and we have a number of reports on the pathologic changes which arise in the internal organs, very little comment has been made on the changes which may be noted in the tissues of the spleen. In the studies which have been carried out on postmortem material the greatest attention has been given to the heart and liver. This might be expected inasmuch as the clinical manifestations arising in yellow fever are, to a great extent, concerned with lesions in these organs, and the most prominent pathologic changes are those which appear in the hepatic tissues. The best discussions on the microscopic pathology of yellow fever have been given us by Councilman,[1] Rocha-Lima,[2] Seidelin,[3] and Marchoux and Simond.[4] It is evident from all of these studies that the injury done to the tissues in yellow fever is dependent on a severe intoxication in which a fatty degeneration and necrosis makes its appearance in the organs. The liver and kidney suffer most severely, and many other tissues are similarly involved but play a minor rôle in bringing about the clinical manifestations. In none of these tissues is there evidence of a primary inflammatory response, nor is it evident from the histologic characters that the degenerative processes are directly referable to the presence of the infectious virus in the involved areas. It would seem that the intoxication which involves the various structures in the body is brought to them by the blood stream, being produced at an undetermined site. Since this intense intoxication often leads, within a few days, to an almost complete necrosis of the liver, it would seem that the poison, as well as the virus, is present in the circulating blood and has its influence on all susceptible organs. This toxin, which has not been isolated, does not produce similar lesions in the experimental animals which have been tested.

* The observations on which this paper is based were conducted under the auspices of the International Health Board.

Received for publication June 10, 1927.

It is the usual finding in yellow fever that the spleen is neither altered in its size, nor are definite macroscopic changes sufficiently pronounced to attract attention. Nevertheless on closer examination the splenic structures are found to have suffered changes which are sufficiently distinctive to be of diagnostic value in distinguishing the lesions from those arising in other tropical diseases with which yellow fever may at times be confused. For this reason, I draw attention to certain alterations which I have observed in the splenic structure in cases of yellow fever occurring in West Africa. It is, however, also important to point out that these changes which appear in the spleen are also encountered in other acute intoxications both bacterial and chemical, in which, however, liver necroses are wanting.

These studies are based on a series of thirty-five cases of yellow fever which came to necropsy in Nigeria and the Gold Coast in 1926 and 1927. We are indebted to Dr. Henry R. Muller of the Yellow Fever Commission, Dr. Andrew Connal, Director of Medical Research, Nigeria, and to Dr. W. A. Young, Director of the Pathological Laboratories, Gold Coast, for placing their materials at our disposal. Of the thirty-five cases, twenty-eight occurred in European whites, and seven in native blacks. Death took place from the third to the tenth day after the beginning of the illness. In all of them the clinical diagnosis of yellow fever was substantiated in the pathologic analysis of the organs.

The macroscopic examination of the spleen offers little evidence of changes occurring within its structure. The spleen is commonly of normal size, usually quite flabby and dark in color. On section it is noted that the pulp is markedly congested and the tissues are quite friable. The malpighian bodies are visible as small greyish areas and may appear more prominent than normal. At other times again, the malpighian bodies are blurred, and rather diffuse in their periphery, having lost their punctate appearance. Necroses are never visible to the naked eye. The appearance of the spleen may be altered by the presence of changes due to chronic malaria, which is usually prevalent in yellow fever areas. However, in those individuals who have recently arrived in the infected zone, and in whom such added lesions are not present, the characteristic lesions are fairly constant.

The microscopic examination shows the sinusoids of the pulp sub-

stance widely dilated and engorged by red blood cells. There is rarely evidence of infiltration by polymorphonuclear leucocytes in the pulp tissues, and we observe an increase in these cells of the spleen only in cases where there is considerable necrosis of endothelial cells following hyperplasia. With the dilatation of the sinusoids, the endothelial cells appear to be crowded along the borders of these channels, and only occasionally are mild reactions of endothelial hyperplasia observed in the pulp areas. These endothelial cells show some phagocytic activity by the presence in their cytoplasm of occasional red blood cells and pink-staining debris from necrotic cells. The numbers of phagocytic cells or of loosely scattered large endothelial cells along the course of the sinusoids are never great.

On the other hand, more definite and constant changes are observed in the lymphoid follicles. These structures appear to pass through a sequence of changes, in the early stages of which there is some increase in the endothelial cells of the germinal centres. The lymphoid follicles of the spleen normally possess a small collection of endothelial cells in their centres, as well as a sprinkling of similar cells through the main lymphoid mass towards the periphery. The early reaction in the moderately intense cases of yellow fever is one in which the follicle appears somewhat enlarged through the hyperplasia of the endothelial elements. This is soon followed by a diminution in the size of the follicle resulting from the loss of the lymphocytes which disappear from the spleen structure entirely. These lymphocytes not only diffuse through the spleen pulp, but also disappear from it so that the spleen is less richly supplied with lymphocytes than normally. Frequently the loss of the lymphoid elements leads to a disappearance of many of the follicular masses, leaving behind patches of endothelial cells. However, when this stage has arrived, evidences of endothelial degeneration are prominent.

Associated with the loss of lymphocytes from the malpighian bodies, the endothelial cells in their peripheral portion and in the pulp immediately contiguous to them, become more prominent. These cells are larger and more numerous than normal; and are arranged along the borders of the vascular spaces or are loosely attached to the neighboring reticulum. Some of the cells appear quite free and show no regular relation to the fixed structures of the part.

These endothelial cells vary somewhat in size; most of them are spherical but some are elongated or distorted. Turnbull,[5] who has also commented upon the presence of these cells, refers to them as free endothelial cells. They possess phagocytic qualities and we have noted that in some cases they suffer a granular and, at times, hyaline degeneration similar to those which lie within the midportion of the lymphoid follicles. A similar finding has been reported by Durham [6] who, in describing the spleen, states that "the marked feature is the large number of active macrophage cells in the pulp; some much vacuolated and most containing remnants of nuclear material."

In the later stages of the reaction, the endothelial cells occurring in the germinal centers of the malpighian bodies show hyaline changes and a fusion of their structures so that little remains of the original germinal centres. Towards the periphery of the follicle the degeneration of the endothelial cells is not of this type, or as marked, but the individual cells show a primary enlargement and granular degeneration of the cytoplasm, with subsequent death of the cells and a formation of protoplasmic debris. This endothelial degeneration in the outer zones of the follicle frequently gives rise to very bizarre types of cells varying from large multinucleate forms to irregular fragmentations which are partly phagocytosed by the neighboring reticulo-endothelium. Associated with these evidences of degeneration in the endothelial cells there are also some cells exhibiting mitoses of their nuclei. The mitotic figures appear as monasters or diasters, but the chromatin material which is arranged in rods appears very dense and without the delicate structure of normal mitoses. Furthermore, these cells exhibit areas of degeneration in their cytoplasm which assume a hyaline and somewhat acidophilic character. It appears to us that these mitotic processes do not indicate a normal process of cell division, but one of nuclear response of a degenerating cell.

The lesions in which these peculiar endothelial changes are most prominent are in the borders of the follicles where the funnel-shaped extremities of the efferent capillaries are arranged about the follicles. These have been described by Robinson.[7]

During the process of degeneration of the endothelial cells and subsequent to the enlargement of their structure, fragmentation of the nuclei gives rise to peculiar chromatin masses which may readily

be mistaken for protozoan parasites. Ring formations like those of malaria, and leishmania types with a false nucleolus are not uncommon, but a differential staining shows these structures as irregular nuclear masses arising through fragmentation. In the advanced stages of the disease the debris arising from the endothelial cells is much increased and can be traced as a zone in the periphery of the follicles.

These reactions of the spleen differ from those of the liver and kidney where the parenchymatous cells alone suffer necrosis while the endothelial cells, though showing fatty degeneration, are not found to suffer complete destruction. During the stages of hyperplasia and early degeneration of the endothelial cells in the spleen, these cells may show the accumulation of fatty substances in their cytoplasm. Up to the present we have not observed similar reactions in the spleen of guinea pigs inoculated with the blood of yellow fever cases. The lesion is quite distinctive from that seen in human cases of relapsing fever.

The reaction arising in the spleen suggests the influence of a toxic agent brought to it by the blood or diffused into its tissues by a virus which is harbored locally. The absence of any evidence of a cellular exudate distinguishes the lesion from infections by the common bacteria. Unfortunately our analyses did not include the examination of the bone marrow to observe whether similar reactions, as those described by Bunting,[8] Jordan,[9] and others, in the megalokaryocytes of the bone marrow, had taken place.

The quantity of red blood cell phagocytosis carried on by the reticulo-endothelial system is never great, and does not play an important rôle in blood destruction in yellow fever.

The only reference which we have encountered which deals with the changes arising within the spleen in yellow fever is that of Turnbull whose observations are very similar to our own. Turnbull, however, was able to carry out his observations on only two cases of yellow fever, and has not had the opportunity of observing the successive changes which arise in the course of the disease. We have found that a careful examination of the spleen is frequently helpful in assisting in the pathologic diagnosis where yellow fever must at times be differentiated from other conditions giving rise to liver and kidney damage.

REFERENCES

1. Councilman, W. T. U. S. Marine Hospital Service, 1890, 151.
2. Rocha-Lima, H. da. *Verhandl. d. deutsch. path. Gesellsch.*, 1912, xv, 163.
3. Seidelin, H. *Yellow Fever Bur. Bull.*, 1915, iii, 269.
4. Marchoux, E., and Simond, P. L. *Ann. de l'Inst. Pasteur*, 1906, xx, 16.
5. Turnbull, H. M. Report of Yellow Fever Commission (West Africa), 1915, i, 200.
6. Durham, H. E. Liverpool School of Tropical Medicine, Yellow Fever Expedition to Parà, 1902.
7. Robinson, W. L. *Am. J. Path.*, 1926, ii, 341.
8. Bunting, C. H. *J. Exper. Med.*, 1909, xi, 541.
9. Jordan, H. E. *Am. J. Anat.*, 1920, xxvii, 287.

LOCAL AND SYSTEMIC CHANGES INDUCED BY LIPOID SOLVENTS IN ANIMALS FED ON DIETS VARYING IN VITAMIN CONTENT *

LOUIS H. JORSTAD, M.D. AND CHARLES G. JOHNSTON, M.D.

(From the Research Laboratories of the Barnard Free Skin & Cancer Hospital, and the Department of Surgery, Washington University School of Medicine, St. Louis, Mo.)

In this paper we will give additional data in regard to the local and systemic changes produced in the laboratory animal as a result of the introduction of any one of several lipoid solvents into the subcutaneous tissue. We have indicated the nature of these local and systemic changes and their relation to different dietaries in a preliminary report.[1]

The great majority of chemical substances that are now known to produce cancer are fat solvents, the same agent producing either carcinoma or sarcoma. That some general change in the organism as a whole regulates the degree of reaction induced about the lipoid solvent in a localized area of tissue and the relationship of this to the formation of malignancy, has been emphasized in former papers from this laboratory.[2,3] Wolbach and Howe [4] have noted that diets deficient in vitamin A cause a transformation of the epithelial layer into stratified squamous keratinizing epithelium in the upper respiratory tract, salivary and accessory salivary glands, later in the pancreas, renal pelvis, bladder, seminal vesicles, epididymis and prostate gland.

In our animals fed a diet rich in vitamin A the local tissue change about the droplets of a lipoid solvent is much different from that in the animals fed balanced vitamin dietaries and diets deficient in vitamin A. The variation of the vitamin B content has little significance except that adequate vitamin B must be present in the diet to balance the vitamin A or the animal will neither live for a long period of time nor have a normal growth curve. On the rich vitamin A diet the lipoid solvent breaks up into numerous small droplets in the local area, and a zone of fibroblasts surrounds each one. There is a certain amount of proliferation as evidenced by the presence of mitoses for a period of thirty days or longer. A tumor persists that

* Received for publication June 17, 1927.

is made up of oil droplets surrounded by a capsule of fibrous tissue showing various grades of hyaline degeneration. Whereas the oil droplets may become partly displaced by the capsule, they never disappear completely, even as long as six to nine months after the injection.

As noted in previous papers, tissue changes about droplets of coal tar may be altered by the diet given an animal. For our largest series of experiments we have used the white rat. For a smaller series we have used monkeys and guinea pigs to corroborate our findings in the rat. The same character of tissue change occurs about droplets of mazola oil, paraffin oil and neat's-foot oil. There is some variation in regard to the degree of change produced by these different substances, but as a class the reaction is the same. The state of nutrition of the animal, especially as influenced by the vitamin content of the dietary, regulates not only the degree of the tissue change about these oils, as about coal tar, but also regulates the manner of change which may take place in these substances themselves. It became evident, therefore, that coal tar and paraffin oils, classed as mineral oils; mazola oil, classed as vegetable oil; and neat's-foot oil, classed as animal oil, act in the same manner on the tissues. Our work on coal tar indicates that this action must be the result of their ability to dissolve lipoid substances from the tissues. It became of interest, therefore, to observe if the same reaction in the animal is brought about by different fractions and different mixtures of these lipoid solvents in the animals fed the different dietaries.

By removing the fats from coal tar with ether, we obtain a black substance that has the gross appearance of soot. It is in fine particles. Making a suspension of this with normal salt solution we injected different quantities into the subcutaneous tissue of rats.

We have used rats for the greater part of our experiments, because this animal does well on a food ration which conforms basically to that of man. Then too, spontaneous tumors are uncommon in the rat and our strain of rats has been inbred over a long period of time, accurate data having been kept of these different strains as to their period of life cycle, fertility and weight curves.

Even on a rich vitamin A ration there is an extensive early complete hyalinization of the tissue about the particles of soot. This soot is evidently more active than crude coal tar because in a previous paper [2] it was shown that coal tar produced a cellular reaction

in rats fed a dietary rich in vitamin A. Hyaline changes occur quickly in animals fed a dietary deficient in vitamin A. From experiments with the tissue culture [5] it has been shown that fats and oils act to remove the ergusia, a lipoid constituent of the cell. Later work [6,7] has shown that the ergusia is either vitamin A or replaced by vitamin A. Burrows [8] has further shown that the chemistry of cells and intercellular substances is such that the removal of the ergusia should cause either hyaline or granular changes. In terms of these analyses we have concluded that the coal tar soot is more active than coal tar in dissolving this fraction.

In other experiments we injected two cubic centimeters of cod liver oil, two of neat's-foot oil, two of mazola oil and two of paraffin oil, subcutaneously in a series of rats fed diets deficient in and rich in vitamin A respectively, with a series on a balanced vitamin dietary and a stock laboratory ration dietary as controls. In every instance the diet rich in vitamin A caused the oil to become broken into many smaller droplets. Around each droplet there is an increase in the number of fibroblasts and less degeneration. In the animals on the vitamin A deficient diet, the lipoid solvent remains in one large droplet and the tissue about it shows marked hyalinization. Coal tar causes the greatest degree of hyalinization, the tissue change about the paraffin, mazola and neat's-foot oil being less in degree but of the same type. In our studies with cod liver oil injected under the skin, we have found that it may form into a cheesy-like mass and does not in all cases become encapsulated like neat's-foot oil or oil extracted from rat tissue. Neat's-foot oil and cod liver oil are animal oils, but no doubt differ greatly in their content of vitamin A. There is no significant difference between the reaction about neat's-foot oil (animal), mazola (vegetable) and paraffin oil (mineral) in the subcutaneous tissue when they are made sterile before injection and introduced under sterile conditions.

Cod liver oil contains large quantities of fat soluble vitamins. In this regard it differs from the oils mentioned above. In this same regard it is interesting that tumors similar to those produced by other oils were seen about droplets of cod liver oil in only two cases out of twenty-five. The relation of this change of reaction to the high vitamin content of this oil is now being investigated.

These studies have given further evidence of the similarity of ergusia to vitamin A. At least they indicate that vitamin A supplies

the ergusia of the cell. As pointed out in another paper [1] coal tar acts most intensely when first introduced. Its action then wanes as any substance that is being used up or is dissolving some fraction from the tissue. In proof that it dissolves a fraction from the tissue, it has been found that it can migrate from the initial point of inoculation after a given period of time. This ability to migrate is the same as noted in fat droplets taken from cells. Burrows [9] found these fat droplets saturated with ergusia. He proved that they were so saturated by showing that they coagulate blood while the same fat as it is fed to animals prevents the coagulation. If the ergusia is the same as one or all of the fat soluble vitamins, then these vitamins should protect the animal against the local action of the lipoid solvent.

The zone of tissue around droplets of mazola oil or coal tar undergoes hyaline degeneration after a certain period of time in the laboratory animal fed a stock ration of dog biscuits, lettuce, meat, corn and water. This hyalinization is similar to that seen in the tissue surrounding a malignant growth. Burrows' work indicates that such degeneration is the result of the removal of the ergusia from the tissue. A different set of conditions takes place when the mazola oil is not allowed to remain in the subcutaneous tissue for a long period after the injection, but is aspirated every two days and fresh oil injected after each aspiration. At the end of thirty days the resultant tumor is made up of cell nests containing many mitoses. The adjacent lymph nodes contain a few oil droplets. At a later period two sets of condition have taken place; there are areas of hyaline tissue and areas made up of densely packed cells. Some of these cell masses have invaded the oil droplet similar to the invasion noted by Burrows and Johnston [9] when Allen-Doisy hormone is added to the mazola oil injected.

The differences in tissue change about these oil droplets in the animals on the various dietaries may be related to the clinical course of oil tumors in man. In different individuals, oil tumors undergo the same variation that we have found resulting from giving closely related animals different dietaries. The oil tumors in one individual may disappear or at least cause no trouble. In another individual they may persist or later spring into activity. That these changes are related to the nutrition of the patient is indicated by a recent case which came to the laboratory. This was a case with multiple

oil tumors in the sheath of the deltoid muscle.[11] These tumors resulted from an oil injection eleven years previous and had caused no trouble until she was placed on a vitamin-free, salt-free, dietary. She was placed on this dietary for the treatment of nephritis. On the salt-free, vitamin-free dietary, the oil tumors increased rapidly in size and became painful, only to recede again to their former size on a vitamin rich dietary. Microscopically these tumors are similar to the tumors produced by the introduction of lipoid solvents into the tissue of the laboratory animal (rat, guinea pig and monkey). The oil has broken up into small droplets. We do not know the nature of this oil, but probably it is mineral or vegetable oil used as a menstruum for the administration of a drug. Each droplet is surrounded by a zone of cellular fibrous tissue containing a few lymphocytes, plasma cells and giant cells. From previous studies on animals this reaction in this patient can be explained as the result of the oil removing the ergusia from cells about it. So long as the individual was on a balanced vitamin dietary the tumor remained quiescent, because the ergusia absorbed by the oil was replenished by the system. When the vitamin A was withdrawn from the dietary an imbalance resulted, the tissue about the oil droplets became deficient in ergusia and as a result the cells were allowed to grow.

In a study of the gross and microscopic sections of the internal organs of the rats injected with these lipoid solvents or mixtures of them, we noted a striking departure from the usual in the series fed on a diet rich in vitamin A. These rats always developed a fatty liver sooner or later. Visible fat in the liver has not been observed as the result of feeding a diet rich in vitamin A. It occurs in these animals only when they are also injected subcutaneously with sufficient doses of one or the other lipoid solvents.

The amount of fat in the rich vitamin A diet is also evidently not concerned with the appearance of fat in the liver. The appearance of fat in the liver was associated with the high vitamin A content of the fat. Diets containing equal quantities of crisco in place of butter were not associated with fatty livers in animals receiving sufficient quantities of lipoid solvents injected subcutaneously.

In our preliminary report [12] we stated that a mixture of paraffin oil and coal tar is peculiar in that it produces a fatty liver. Our later work has indicated that this is not true. Any lipoid solvent injected in sufficient quantities subcutaneously will produce this change in

the liver. The mixture of paraffin oil and coal tar acts more quickly than either of these oils alone, or other oils we have used. The interesting fact is that the different oils and mixtures of oils have each their own time interval between their injection and the appearance of the fatty liver. The mixture of coal tar and paraffin oil produces this change in 30 days after a single injection of two cubic centimeters, the same quantity of a mixture of coal tar and mazola oil produces this change after 40 to 60 days, mineral oil alone after 45 to 60 days, mazola oil alone after 60 to 90 days, and coal tar alone after 90 days. The growth curves of the rats in this series are interesting, in that they indicate the time of the appearance of fat in the liver. There is always a transient decline in weight preceding the appearance of fat in the liver.

The livers of these rats were studied not only grossly but microscopically. The liver of the rats fed on a high vitamin A diet and injected with the lipoid solvents contained very large quantities of fat which stained sharply with Sudan III. The fat was contained in large irregular vacuoles, which had smooth contours. The remainder of the liver cells were well preserved. These livers did not resemble in any way the necrotic fatty livers produced by injecting chloroform, phosphorus, etc.

To determine the relationship, if any, between the lesion we observed in the above series and that produced by chloroform, a series of rats were placed on rations containing different quantities of vitamin A, different ratios of vitamins A and B, and different ratios of carbohydrate, protein and fat. It has been well established by Opie and Alford [13] and others, that the toxic effects of chloroform on the liver can be prevented to a certain degree by a high carbohydrate diet. In a part of our series the chloroform was added to a lipoid solvent (mazola oil, paraffin oil or neat's-foot oil) and the mixture injected into the subcutaneous tissue according to the technic used in the production of oil tumors described by us in former studies. In a part of the series the chloroform was administered by inhalation. The hepatic lesion in these animals is a necrosis associated at times with a small amount of fatty infiltration. The picture is different from that of the above series, the chloroform lesion being one of destruction, the coal tar, paraffin, mazola lesion being one of fatty infiltration in a cell with intact nucleus. In corroboration of the work of Opie and Alford and others, we found that a high

carbohydrate diet has a protective action against chloroform necrosis of the liver. A diet rich in vitamin A, the carbohydrate being low or moderate in amount, has no influence on these toxic effects. However, a diet rich in both carbohydrate and vitamin A protects the rat to a greater degree than a diet high in carbohydrate and not rich in vitamin A.

Since the livers from the rats injected with the lipoid solvent and given the high vitamin A diet show no destructive lesions in the liver microscopically, the presence of fat must be due to an accumulation of fats from the blood stream. The body as an organism may be considered as essentially an aqueous solution containing proteins, fats, carbohydrates and salts. As Burrows has demonstrated with the tissue culture [10] the process governing the migration of fat droplets is not different fundamentally from that governing the migration of body cells. A droplet of fat will migrate from an area containing less to an area containing more ergusia. The droplet will remain stationary when it is saturated to the same degree as the environment about it. That the liver should become saturated with fat droplets in an animal fed a ration rich in vitamin A and not in an animal fed a dietary deficient in vitamin A when a lipoid solvent has been introduced locally into either of such animals is readily understood when we consider the migration of fat to be such a simple process.

Because the lesion in the liver produced by chloroform is that of necrosis and that produced by mazola, paraffin and coal tar is one of infiltration, it does not mean that these substances are not alike in their action. Coal tar, mazola oil and paraffin oil are not absorbable, they remain probably indefinitely, in part at least, in the area of tissue into which they have been injected. They remain as encapsulated tumor masses. In this local area they absorb slowly the ergusia from the tissues for a long time. In a system saturated with ergusia they cause enough disturbance of the ergusia balance so that the liver becomes filled with fat. The liver is much richer in ergusia than the subcutaneous tissue of the body, and especially the subcutaneous tissue from which a certain amount of ergusia has been removed by the lipoid solvent.

Chloroform is readily absorbed and is a more active lipoid solvent than the mineral, vegetable or animal oils. The necrosis of the liver occurs after introduction of the chloroform into the subcutaneous

tissue or after inhalation of small amounts. The necrosis occurs even as soon as twenty hours after the inhalation or introduction of chloroform into the subcutaneous tissue. No doubt the whole body is saturated with the chloroform at this period of time. Inasmuch as the liver is presumably the site of highest concentration of ergusia in the body, much of the chloroform should be transported there early. Thus, the chloroform in the liver acts as coal tar in the subcutaneous tissue. Coal tar produces a certain amount of degeneration in the areas about it. Chloroform is a more drastic lipoid solvent. It removes more of the ergusia from the liver parenchyma with a resultant precipitation of the cell proteins and the typical picture of necrosis. With small doses of chloroform given to rats on a *protective* dietary, the lesion is periportal, it is around the blood vessels which no doubt carry the chloroform to the liver. If a *non-protective* dietary or larger quantities of chloroform are used, the whole liver undergoes necrosis, the animal dying as a result.

Discussion

In previous experiments [2] it was found that coal tar produces both local and systemic changes when introduced into the tissue. It was shown also that the systemic change resembled grossly the cachexia of malignancy. These systemic and local changes vary with the vitamin content of the dietary given the animal, the carbohydrates, proteins, fats and salts being kept more or less constant. In these studies we have found that other vitamin free oils, animal and vegetable in nature, produce the same changes and respond in the same way to dietary.

In making these studies we have observed another interesting systemic change. There is an accumulation of fat in the livers of rats fed a ration high in vitamin A and injected with a lipoid solvent. We have not noted these changes in rats fed a ration high in vitamin A alone. The time of occurrence of this fatty infiltration varies with the oil or combination of oils used.

The results of the earlier experiments with coal tar were explained by assuming that the coal tar dissolved the ergusia from the tissues as Burrows had shown in the tissue culture. The fat soluble vitamins protected the animal against these effects by restoring the ergusia dissolved. These later observations can be explained as the

result of a migration of fat from the tissues to the liver. This migration results because in the animals fed a high vitamin A diet the liver must be rich in these vitamins. The tissues on the other hand are depleted of them by the lipoid solvent injected.

When vitamin A is added to the system the lipoid solvent remains active in the subcutaneous tissue for a long period of time. Fibrous tissue forms around it, the ergusia and archusia in the area become balanced and hyalinization is prevented, due to the fact that the animal supplies the ergusia which is being removed by the lipoid solvent. The animal is able to supply this ergusia because it is getting a large amount of vitamin A in its dietary. The lipoid solvent absorbs ergusia until it is saturated, then breaks up into droplets and migrates to other organs of the body. We have noted droplets of lipoid solvent in the lungs, in the liver and in the tissues a considerable distance from the oil tumor.

The variation in the amount of hyalinization in the tissue about the droplets of lipoid solvent is a factor regulated apparently by the relative amount of ergusia and archusia in the tissue. In earlier studies [2] the variation of this factor was noted. There is an increased hyalinization of tissue about droplets of coal tar in the subcutaneous tissue of an animal fed a ration high in vitamin B. This is a system rich in archusia. This hyalinization was not so marked as that occurring about droplets of coal tar in an animal on a dietary deficient in vitamin A. This is a system low in ergusia and consequently high in archusia. The type of hyalinization is similar to that seen in areas surrounding malignant growths in man. Former studies of Burrows and Jorstad [7] have shown that malignant tissue is very high in archusia. This area high in archusia is depleting the surrounding tissue of its ergusia with the resultant hyalinization taking place.

Considerable data have been published recently in regard to the availability of the vitamin A in the system, especially as influenced by the intake of vitamin B, or at least the concentration of vitamin B in the system. Collazo and Funk [14] state that one cubic centimeter of autolyzed yeast permits the highest food intake on a diet with the ratio of carbohydrate to protein 8:1. This ratio corresponds to that found in cereal grains. Agduhr and Höjer [15] have recently shown that an excess of cod liver oil in the dietary may cause ill effects. They find vacuolar degeneration and brown atrophy of the heart muscle in these cases. Agduhr [16] concludes that these

changes are caused by some injurious substance in the cod liver oil. Höjer [17] records experiments on rats proving that they are not produced by a poison in the oil, but are produced by an overdose of cod liver oil without simultaneously increasing the vitamin B in the food. We have noted no changes in organs other than the liver.

Plimmer and Rosedale [18] first drew attention to the fact that the ill effects of large doses of cod liver oil could be overcome by increasing the quantity of vitamin B in the food. Their experiments were done with chicks. Studies in this laboratory show quite clearly that the same is true of overdoses of vitamin B. The ill effects of an overdose of vitamin B, whether it is given in the form of a commercial product, such as Marmite (vegex), autolyzed yeast, X-rays [19] or young bacterial cultures,[7] may be prevented to a certain extent by the addition of adequate vitamin A in the dietary. It is when there is a proper balance between these two vitamins that a functioning mechanism results.[20]

Levine and Smith [21] in a recent report state that the livers from rats fed a dietary containing as high as 86 per cent of the total calories in the form of fat, provided the protein and salt requirements of the animal are fulfilled, contain no fat droplets when examined microscopically.

Conclusions

1. Various oils and fats act like coal tar when injected into the subcutaneous tissue of animals. All of them dissolve the lipoids of the cell and may be classed as lipoid solvents.

2. The cellular activity about droplets of various lipoid solvents injected into the subcutaneous tissue of rats is greatly increased by feeding a diet rich in vitamin A.

3. The lipoid solvent remains *en masse* in an animal fed a ration deficient in vitamin A and disperses into smaller droplets in the animal fed a ration rich in vitamin A.

4. The tissue reaction about the lipoid solvent is altered in degree if the fats are removed from the solvent with ether before it is injected into the tissue.

5. Rats injected subcutaneously with certain lipoid solvents or mixtures of them and fed a diet rich in vitamin A suffer a massive fatty infiltration of the liver. This infiltration occurs at different periods of time after the injection of the different lipoid solvents.

6. Chloroform produces a necrosis of the liver because it diffuses rapidly throughout the system. This lesion is similar to that produced locally by coal tar in the tissue.

REFERENCES

1. Jorstad, L. H., and Johnston, C. G. *Proc. Soc. Exper. Biol. & Med.*, 1926, xxiv, 86.
2. Jorstad, L. H. *J. Exper. Med.*, 1925, xlii, 221.
3. Jorstad, L. H. *J. Cancer Research*, 1926, x, 229.
4. Wolbach, S. B., and Howe, P. R. *Proc. Soc. Exper. Biol. & Med.*, 1925, xxii, 402.
5. Burrows, M. T. *Proc. Soc. Exper. Biol. & Med.*, 1924, xxii, 241.
6. Burrows, M. T., and Jorstad, L. H. *Am. J. Physiol.*, 1926, lxxvii, 38.
7. Burrows, M. T., and Jorstad, L. H. *Am. J. Physiol.*, 1926, lxxvii, 24.
8. Burrows, M. T. *J. Cancer Research*, 1927, xi, 72.
9. Burrows, M. T., and Johnston, C. G. *J. Exper. Med.*, 1925, xlii, 215.
10. Burrows, M. T. *Am. J. Anat.*, 1926, xxxvii, 289.
11. Burrows, M. T., and Jorstad, L. H. *J. A. M. A.*, 1927, lxxxviii, 1460.
12. Jorstad, L. H. *Proc. Soc. Exper. Biol. & Med.*, 1927, xxiv, 723.
13. Opie, E. L., and Alford, L. B. *J. A. M. A.*, 1914, lxii, 895.
14. Collazo, J., and Funk, C. *J. Metab. Research*, 1924, v, Nos. 4, 5 and 6.
15. Agduhr, E., and Höjer, A. *Lancet*, 1927, ccxii, 195.
16. Agduhr, E. *Acta Pediat.*, vi, Fasc 2, 165.
17. Höjer, A. *Acta Pediat.*, vi, Fasc 2, 180.
18. Plimmer, R. H. A. *Proc. Roy. Soc. Med.*, 1926, xix (Sect. Compar. Med.), 21.
19. Burrows, M. T., Jorstad, L. H., and Ernst, E. C. *Radiology*, 1926, vii, 279.
20. Burrows, M. T., Jorstad, L. H., and Ernst, E. C. *J. A. M. A.*, 1926, lxxxvii, 86.
21. Levine, H., and Smith, A. H. *J. Biol. Chem.*, 1927, lxxii, 223.

THE EFFECT OF AGE OF THE HOST ON THE FATE OF TRANSPLANTS OF THYROID GLANDS IN GUINEA PIGS *

LOUIS LEON TUREEN

(From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.)

In these experiments, an attempt was made to determine what effect, if any, the age of the host, as exemplified by guinea pigs, had upon the reaction, towards homoiotransplanted thyroid gland. Male adult animals, weighing 500 to 700 gm. were used as donors. In some instances, the two lobes of thyroid were transplanted simultaneously, one into a female guinea pig and the other into one of the litter. In the majority of cases, the adult hosts were females, 500 to 800 gm. in weight, while the young, the age of which varied between four days and three weeks, and which weighed on the average 150 gm. were of either sex.

The technic used in these experiments was the same as that employed in previous experiments of this series of transplantations. The lobes of the thyroid were placed in subcutaneous pockets made in the abdominal wall. Two sets of experiments were carried out. In one, the transplants were removed for examination at intervals varying between ten and twenty days after transplantation, while in the other series the interval was twenty days. The former experiments had already been done by Dr. Leo Loeb [1] and have been referred to by him in a previous publication.

The typical reaction to homoiotransplants in general has been described in the papers of Loeb and his associates [2] and Hesselberg,[3] especially, as far as the thyroid gland is concerned. In the first four or five days after transplantation there is practically no difference in the reaction to auto- and to homoiotransplantation. During the first day or two, large central parts of the gland become necrotic. After forty-eight hours, the first fibroblasts, a few polymorphonuclear leucocytes and lymphocytes surround the graft, and appear in the center of the transplant in seventy-two hours. In four days, regeneration of acini begins, the necrotic center becomes smaller, the number of fibroblasts and lymphocytes in the necrotic part in-

* Received for publication June 6, 1927.

creases, and new vessels become visible. The first difference between auto- and homoiotransplants to appear consists in an increase in the number of fibroblasts and lymphocytes in the homoiotransplants. In the latter, between the seventh and twelfth day, the fibrous tissue invasion becomes very intense in typical cases. Thus, individual acini and groups of acini are surrounded and destroyed by fibrous bands. The difference between auto- and homoiotransplants is especially marked in the centers of the transplanted pieces, which, in cases of the latter kind of transplant, are organized by dense connective tissue in which the vascularization is poorer than in autotransplants. In the homoiotransplants, lymphocytes are present in large numbers; by direct invasion and compression they destroy the acini and, having penetrated their lumen, destroy the colloid as well. On the other hand, wherever the thyroid tissue has not perished from the action of connective tissue or lymphoid elements, it is as well preserved as in the autotransplant. It may therefore be assumed that the destruction of the homoiotransplant is largely due to the action of lymphocytes and fibroblasts. In the majority of cases the thyroid is attacked with so great an intensity that at the end of twenty days there is little or no thyroid tissue left at this time, although the date of complete destruction varies in individual cases. The fate of the average homoiotransplant of thyroid gland in adult hosts is, therefore, a gradual disappearance of the gland tissue, and its replacement by connective tissue.

By varying the ages of the hosts in different experiments, we wished to determine in what manner the age of the host affects the character of the homoio-reaction just described. The effects of the age factor upon the degree of preservation of thyroid acini, on the colloid and on the intensity of lymphocytic and connective tissue reactions, were particularly studied. As stated above, in some cases, thyroid lobes were transplanted from the same donor simultaneously into mother and offspring. In such cases, the transplant into the mother served as control for the lobes transplanted into the young guinea pig. In other instances, pieces of thyroid transplanted into other adult animals were used as controls. In our first series, transplants into 20 adults and 20 young animals, and in the second, transplants into 34 adults and 25 young animals were examined; a total of 99 transplants, of which 54 were from adult, and 45 were from young hosts. On the whole, the results showed a better degree of

preservation of thyroid tissue in young animals than in the adult hosts, though the longer the transplant remained in the host, whether old or young, the greater was the tendency towards destruction of the glandular tissue.

We shall consider first the fate of transplantations into adults, in both series. In the first series, we found seventeen pieces of thyroid tissue, or 85 per cent of the transplants into adults, showing little or no preservation. Included among these cases were eight pieces which showed poorer preservation than those from corresponding young animals, two pieces in which destruction was complete in both old and young and seven cases of practically an entire disappearance of the thyroid gland in the adult, while in the average of the young hosts, which served for comparison, the preservation was better. In these transplants into adult guinea pigs, fibrous tissue and lymphocytes were much in evidence, though an occasional acinus or peripheral group of acini containing colloid, could be found. However, there were three transplants into adult hosts in which the thyroid tissue was relatively well preserved. One, a fourteen day transplant, showed so little reaction that it resembled an autotransplant. In the other two, which were likewise fourteen day transplants, the number of preserved acini was smaller, though in the periphery of the transplant they were more numerous and larger than those usually found in this kind of homoiotransplantation. However, a rather marked lymphocytic and connective tissue reaction was noticeable which separated and destroyed the glandular elements.

In the second series, the twenty day transplants, twenty-seven or almost 80 per cent of the transplants into adults showed practically a complete destruction of the thyroid gland, with replacement by dense fibrous tissue and with a greater or less degree of lymphocytic infiltration. In some instances an isolated, collapsed or fragmented acinus, free from colloid, was present. Of the remaining twenty day transplants, one (Fig. 5) showed a peripheral ring of well preserved acini, containing deeply stained colloid, with only a comparatively mild lymphocytic and fibroblastic reaction. This picture strikingly resembles a syngenesiotransplant. Another showed a fairly well developed ring of preserved acini containing colloid with an extremely severe lymphocytic reaction. In the other four, a few acini were found varying greatly in size and in quantity of colloid; in two of

these latter transplants there was a marked lymphocytic reaction, whereas in the other two, the number of lymphocytes was small. At the same time a large quantity of dense connective tissue had been formed in which only isolated islands of thyroid tissue were left. One transplant of fifty days still showed small acini with colloid and an infiltration by a dense mass of lymphocytes.

As an example of typical findings after transplantation of thyroid gland into adult hosts we cite the following protocol of an experiment:

Experiment I. Guinea pig No. 209; weight 560 gm. Transplant removed for examination twenty days after operation. The piece is well encapsulated by fibrous tissue that shows numerous connective tissue cells among the regularly arranged collagen fibers. The peripheral capsule contains large numbers of lymphocytes that accumulate in groups, and appear especially within the capsule and along its internal border. Along the outer margin of the capsule and occasionally enclosed within it, are seen some fat cells. The center of the piece is well organized by connective tissue containing relatively few fibroblasts, a few capillaries and very few lymphocytes; both lymphocytes and connective tissue cells are more numerous toward the periphery and less numerous toward the center. The blood vessels pierce the capsule, enter from the periphery, branch and penetrate toward the center. Clusters of cells scattered toward the periphery consist essentially of collapsed acini or parts of acini, none of which contains colloid, and epithelial cells occurring singly or in isolated groups. These are surrounded by lymphocytes that are wedged between adjacent acini. The thyroid cells are large and vesicular, round or oval. In the interstices between these islands of cells and elsewhere, the transplant is organized by fibrous tissue. (Fig. 1.)

While the preceding record represents the typical finding in the case of transplantation into adult guinea pigs, the following is the protocol of an infrequent case in which the preservation in the adult host is much better than in the average experiments.

Experiment VII. Guinea pig No. 53; weight 695 gm. Transplant removed after twenty days. Weight of animal at end of experiment, 745 gm. A thick, fibrous capsule encloses the tissue. The preservation of thyroid in this case is unusually good; large and small acini, filled with colloid, being numerous. The largest acini are situated

toward the periphery. In the center the acini have been replaced by loose connective tissue in which some fibroblasts and lymphocytes are seen. Lymphocytic infiltration is comparatively slight throughout. Many lymphocytes are found within the acini, partaking in the destruction of the colloid, which in places is retracted or shows vacuolization. The acini vary in shape, apparently in consequence of compression by the surrounding invading cells which in places separate the acini widely. The connective tissue reaction is not marked, though the center shows signs of organization. Blood vessels also enter the central part. At one pole, a rather dense fibrous band isolates an island of acinar tissue from the rest of the gland. (Fig. 5.) The picture of this transplant so resembles that found in the case of syngenesiotransplantation as to suggest that the donor and host in this experiment were related.

Considering next the fate of transplantations into young hosts in both series, we may state that it is more common to find a more or less complete peripheral ring of well preserved, large, colloid containing acini still present at the end of twenty days. In the first series, in which the pieces were removed ten to twenty days after transplantation, fifteen pieces, or 75 per cent of the transplants, show such a preservation, whereas of the remaining five, one, a twelve day transplant, has only a few acini and they are free from colloid; another, a sixteen day transplant, has only a few acini but all of these contain colloid. In another experiment in which only scar tissue is found, the possibility of infection must be considered. Of the remaining two transplants, one shows a somewhat better preservation than is found in the corresponding adult host; while in the other, a severe reaction on the part of the connective tissue, with the formation of fibrous tissue similar to the reactions in the adult controls, has taken place.

In the second series, in which examination took place after twenty days, fourteen cases, or 56 per cent of the transplants, show a good preservation of acinar tissue. Rather sharply contrasted with the practically complete destruction of thyroid observed in typical instances of transplantation into adult hosts which served as controls, the transplants into the young show, on the whole, a better preservation than is found in the best cases in the adult hosts. In one instance in which preservation in the young might be termed as only fair, merely a limited number of small acini being left, the piece from

the adult control shows almost complete replacement by fibrous tissue. On the other hand, in eleven cases, or 44 per cent of the animals included in this series, the picture differs little, if at all, from that found in about 80 per cent of the transplantations in the adult host. In fact, in a few instances, the degree of scarring is much more marked in the young than in the adult controls. (Fig. 7.) There are a number of transplants omitted from consideration because it is quite evident on examination that infection has taken place and destroyed the thyroid with replacement by connective tissue. In these cases, accidental factors vitiate the result. In another instance, the transplant appears to be entirely hemorrhagic, without showing the usual lymphocytic reaction, though organization of the hemorrhagic area has begun. (See Table I.)

TABLE I

Showing the frequency of thyroid preservation

Degree of preservation	20 Day Transplant		10–20 Day Transplant	
	Adult Guinea Pig	Young Guinea Pig	Adult Guinea Pig	Young Guinea Pig
Good	2 (6%)	13 (52%)	1 (5%)	15 (75%)
Medium	5 (14%)	1 (4%)	2 (10%)	0
Poor	27 (80%)	11 (44%)	17 (85%)	5 (25%)
Total	34	25	20	20

In analyzing the results of these transplantations, we compared the intensity of the lymphocytic and connective tissue reactions in the young and adult hosts. In the cases in which the preservation of the tissue in the young is not good, we could distinguish three groups of transplants: (1) In the first group there was a more marked lymphocytic reaction and a less dense connective tissue formation in the young than in the adult host. (2) In the second group the intensities of reaction in both were equal. (3) In the third group, in the adult, the reaction was typical, whereas in the young the transplant had been replaced by a scar without noticeable lymphocytic reaction. However, the number of transplants from young hosts belonging to these three groups was relatively small. In general, if we consider only those transplants in which very little or no tissue is

preserved, there is little difference in the intensity of the lymphocytic and connective tissue reactions in transplants from young and adult hosts. Indeed, there was, perhaps, a greater density of the connective tissue in the young. However, if we compare the transplants from the young, which show a relatively good preservation, with the typical transplants from adult hosts, we find the connective tissue reaction less marked in the young than in the adult, whereas the lymphocytic reaction, on the contrary, in such cases is much more marked in the young than in the adult hosts. It can readily be understood that the thyroid tissue which remains in the young, being greater in amount, is able to attract a larger number of lymphocytes than the few scattered acini which survive in the adult hosts.

The following protocol may serve as an example of a typical transplantation in the young, in which the preservation of the tissue is relatively good:

Experiment I. Guinea pig No. 235; weight 125 gm. Age less than three weeks. Transplant removed after twenty days. This young guinea pig, and the corresponding adult guinea pig No. 209 (Fig. 1) each received one lobe of thyroid from the same donor, the transplant in the adult serving, therefore, as a control for the tissue obtained from guinea pig No. 235. There is a very thin capsule surrounding the transplant in the young animal. In the periphery there is a ring of acini containing colloid, the acini being largest towards the periphery, while the smaller follicles are more centrally located. A large vessel enters from the periphery, giving off branches along its course. Blood and lymph capillaries seem to be most numerous in the center where organization of the transplant by connective tissue is most complete. In many places, lymph vessels, filled and surrounded by lymphocytes, may be seen. In the central, dense, fibrous tissue there are few cells. Strands of collagen fibrils ramify throughout the transplant. In a middle zone between the center and the peripheral acinar ring, many lymphocytes, but no normal acini are seen. However, there may be found an occasional, collapsed acinus surrounded by lymphocytes. In general, connective tissue and lymphocytes are abundant in the transplant. The peripheral ring of acini is interrupted in places, the acini here being replaced by masses of lymphocytes. In other places, individual lymphocytes invade the acini, enter between the acinar epithelial cells, appear in the colloid and subsequently destroy the latter, as evi-

denced by the presence of vacuoles in the colloid and numerous lymphocytes in the acinar lumen. More peripherally there are large, uninjured acini, with retracted and normal-appearing colloid. The entire transplant is surrounded by a fibrous capsule. (Fig. 3.)

As an example of the less frequent case of complete destruction of the transplant in a young host, the following protocol is presented:

Experiment XLII. Guinea pig No. 456; weight 158 gm. Age less than three weeks. Transplant removed in twenty days. The picture is one of complete replacement of the transplant by connective tissue. A peripheral capsule of young fibroblasts surrounds a denser central area. In the center are a number of typical epithelial pearls, the centers of which are completely hyalinized, whereas their peri-

TABLE II

*Showing the Intensity of Lymphocytic and Connective Tissue Reactions * Twenty Day Transplants*

	Lymphocytic reactions	Connective tissue reactions
Stronger in adult hosts	5	11
Stronger in young hosts	15	4
Equal in adult and young hosts	2	4

* This table includes transplants with and without preservation.

phery is composed of squamous epithelium. Very sparse scatterings of lymphocytes are seen. No thyroid tissue is found anywhere. (Fig. 7.)

The twenty day transplants are tabulated according to the relative degrees of lymphocytic and connective tissue reaction. The figures show, in general, that the connective tissue reaction is stronger in the adult hosts, whereas in the younger hosts, at the end of the twenty day period, the lymphocytes are more numerous.

In agreement with the classification of Loeb,[1] we may distinguish three classes of transplants according to the severity of the reaction on the part of the host. In Type I, no thyroid is left; there is merely a replacement of the transplant by connective tissue and a few lymphocytes (Figs. 1 and 7). In our experiments, most of our transplantations into adults fall into this class. Type II shows only a few

groups of acini left within dense fibrous tissue. The center of the piece consists of dense connective tissue; lymphocytes are numerous, and most of the acini are collapsed and without colloid. Some small acini do contain soft colloid or desquamated epithelial cells. There are perhaps three transplants into adults which can be included in this group. In Type III, we find, in certain parts of the transplant, conditions similar to those characteristic of Type II; there is marked fibrosis, the acini are collapsed and free of colloid; lymphocytic infiltration is dense, but other parts show groups of acini which are close together, without fibrous tissue intervening between them. There may be medium and even large-sized acini in the periphery, with colloid in the process of absorption. Lymphocytes surround the acini, and act apparently as agents of destruction. Of this type there are four cases among the transplantations into adults. Such results are occasionally found in any fairly large series of homoiotransplantations. They seem to be due to a similarity of the individuality differentials of hosts and donors, which should be expected in some cases in accordance with the law of probability. Such similarity would tend to diminish the intensity of the lymphocytic infiltration or to delay its appearance, consequently delaying the rapidity of destruction of the thyroid transplant. Had the transplant been left in the host for a longer period of time, as for instance, forty or fifty days, it is not unlikely that it would have been entirely destroyed, since Type III is not commonly found at so late a date. In addition, we observed two or three cases in which preservation was even better than that which corresponds to Type III. There was only a mild connective tissue reaction. The center consisted of loose connective tissue and few lymphocytes. A complete peripheral ring of well preserved acini was present, the latter lying close together and containing good colloid. (Fig. 5.) This picture corresponds to that described by Loeb [4] as Grade V and as the reaction characteristic of a syngenesiotransplant. We may assume that in all probability in such cases there has been a relationship between the donors and the hosts and that we have therefore to deal in reality not with homoiotransplants but with syngenesiotransplants.

Discussion

In typical homoiotransplantations, the connective tissue reaction is usually completed in a period of from seven to twelve days following the operation, while the lymphocytes, the presence of which depends upon the formation of homoiotoxins induced by the difference in the individuality differentials in host and transplant, are usually few or lacking at this time. They usually appear somewhat later, the time of appearance depending on the difference between the individuality differentials. The greater this difference, the sooner the lymphocytes appear and the more numerous they are at a certain time. However, the intensity of the lymphocytic reaction must also depend upon the size and functional efficiency of the living portion of the transplanted tissue after a given period. If, as a result of accidental conditions or a very marked degree of incompatibility between the individuality differentials, only small amounts of tissues are left, the lymphocytic reaction may be relatively slight. In the typical homoiotransplantations we find usually a combination of marked connective tissue and lymphocytic reaction in and around the transplant in adult hosts, but this is found in only about 35 per cent of all transplants into young animals. In the majority of the latter, although the lymphocytic reaction is present, it is very intense only in the twenty day pieces, whereas the connective tissue reaction seems to be absent or very slight in these cases. The characteristic infiltration of homoiotransplants by connective tissue fails to appear in the young hosts in a large proportion of cases which seems largely responsible for the preservation of so much glandular tissue. Instead of a dense central fibrosis with interlacing connective tissue bands extending throughout the specimen, there is usually found, after transplantation into young animals, only a small central plug of fibrous tissue surrounded perhaps by some loose edematous connective tissue and scattered bands of connective tissue. This condition exists in a large number of transplants into young hosts. No dense fibrous tissue surrounds or compresses the acini, but the destruction of the latter, when it occurs, is accomplished largely by the lymphocytes which appear in dense masses in response to the large quantity of preserved glandular tissue. There is thus a great similarity between the character of these transplants and the moderately well preserved tissue of syngenesiotransplants. In both, the

characteristic features consist of a very slight connective tissue reaction combined with delayed lymphocytic infiltration. In their various series of auto-, syngenesio-, homoio- and heterotransplantations, Loeb [4, 5 and 6] and his collaborators, especially Myer,[7] Hesselberg [3] and Sale,[8] have shown that in general, a definite and early connective tissue reaction indicates a marked divergence of the individuality differentials of host and donor, whereas a delayed lymphocytic reaction signifies less divergence.

It is generally assumed, though apparently without definite experimental basis, that in young individuals, tissue transplants find a better soil than in older individuals. In the case of tumor transplantations, the number of takes seem to be greater in younger than in older individuals; at least this is true in the case of certain transplantable tumors. However, the conditions existing in tumor transplantations are not in every respect the same as those in ordinary tissue transplantations. In this connection it may be of interest to point to the experiments of Murphy [9] who found that heterotransplantation of tumors and also of tissues may succeed, at least temporarily, in the allantois of chick embryos but simultaneous transplantation of spleen tissue causes a reaction to take place against the transplant. In our experiments we compared the fate of transplants of ordinary tissue, like thyroid, in young hosts who have passed the nursing stage, and in adult hosts of the same species, and we found distinct differences in the reactions between younger and older hosts. In the latter it is especially the connective tissue reaction on the part of the host which is more pronounced than in the younger hosts; while the lymphocytic reaction may at a certain period be even more intense in the young hosts, owing apparently to the fact that in the young the lessened intensity of the connective tissue reaction, or its absence, permits the preservation of a greater part of the transplanted tissue, which thus is able to call forth the lymphocytic reaction. When enough tissue has been preserved in the older host, the lymphocytic reaction also may be quite pronounced. This separation between the connective tissue and lymphocytic reaction is in agreement with the earlier observation of Loeb on the behavior of syngenesio- and homoiotransplants. In the former, he found the connective tissue reaction to be relatively mild or lacking altogether, whereas the lymphocytic reaction may become very intense at a later date. It is possible that in addition to the

greater intensity of the reactions on the part of the connective tissue of the host and under certain conditions also of the lymphocytes, there may be a greater direct injurious action of the body fluids of the older host on the transplant. However, since the injurious reactions on the part of the host tissues against the transplants are so marked in the older animals, it is difficult to prove that the latter factor plays a significant rôle in the destruction of the homoiotransplant.

As to the mechanism underlying the greater intensity of the reaction on the part of the adult host, we may assume that the individuality differential is as fully developed in the young as in the older host, but that the latter has developed mechanisms which insure a more marked reaction on the part of the host against the strange transplant, whereas in the young hosts these mechanisms are still in a more rudimentary state. Thus in the young hosts often a syngenesio-reaction is obtained instead of the typical homoio-reaction of the adult host.

Summary

If we compare the reactions of adult with those of young guinea pigs against homoiotransplanted thyroid gland we find a more pronounced average reaction on the part of the adult hosts. This reaction manifests itself principally through the early and intense ingrowth and fibrous transformation of connective tissue cells, which exert a destructive effect on the transplant. There may, in addition, be a marked lymphocytic reaction in either host but, in the adult host as a result of the early destruction of great parts of the transplants, the remaining tissue cannot subsequently produce a quantity of homoio-toxins sufficient to attract large masses of lymphocytes around and in the transplant. Now, in the young host the transplanted tissue is still preserved in larger amount and is therefore functionally more active; in this case, the lymphocytic reaction may at a somewhat later date following transplantation be more intense in the young than in the adult host. Homoiotransplants in the young may thus resemble in certain cases syngenesiotransplants in the adult.

REFERENCES

1. Loeb, L. *Am. J. Path.*, 1926, ii, 99.
2. Loeb, L. *J. A. M. A.*, 1915, lxiv, 726.
3. Hesselberg, C. *J. Exper. Med.*, 1915, xxi, 164.
4. Loeb, L. *Am. J. Path.*, 1927, iii, 29.
5. Loeb, L. *J. Exper. Med.*, 1920, xxxi, 765.
6. Loeb, L. and Harter, J. S. *Am. J. Path.*, 1926, ii, 521.
7. Myer, M. W. *Arch. f. Entwcklngsmechn. d. Organ.*, 1914, xxxviii, 1.
8. Sale, L. *Arch. f. Entwcklngsmechn. d. Organ.*, 1913, xxxvii, 248.
9. Murphy, J. B. Monographs of the Rockefeller Institute, No. 21, 1926, 7.

DESCRIPTION OF PLATE

PLATE 128

FIG. 1. Experiment I, Guinea Pig No. 209. Low magnification showing the typical reaction to homoiotransplanted thyroid gland in adult animals after twenty days.

FIG. 2. Higher magnification of the peripheral part of the section shown in Fig. I.

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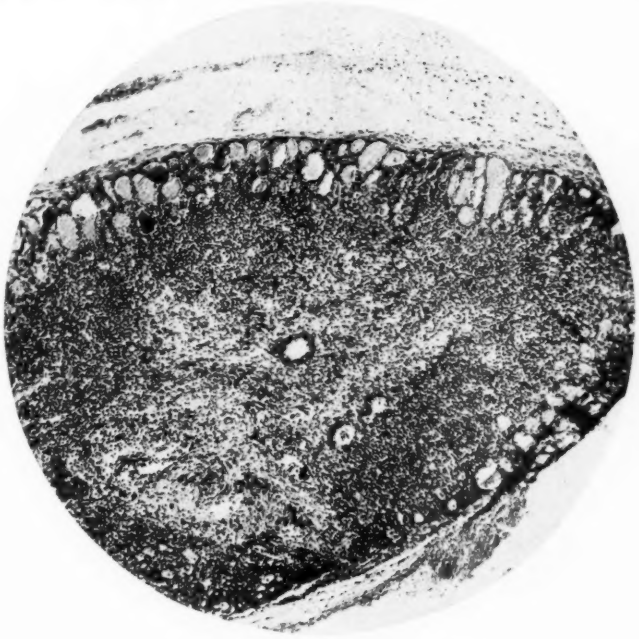
Transplants of Thyroid Glands in Guinea Pigs

PLATE 129

FIG. 3. Experiment I, Guinea Pig No. 235. Low magnification, showing preservation of a peripheral ring of acini in homoiotransplanted thyroid gland in a young animal after twenty days. Both transplants shown in Figs. 1 and 3 were taken from the same donor.

FIG. 4. Higher magnification of the peripheral part of Fig. 3.



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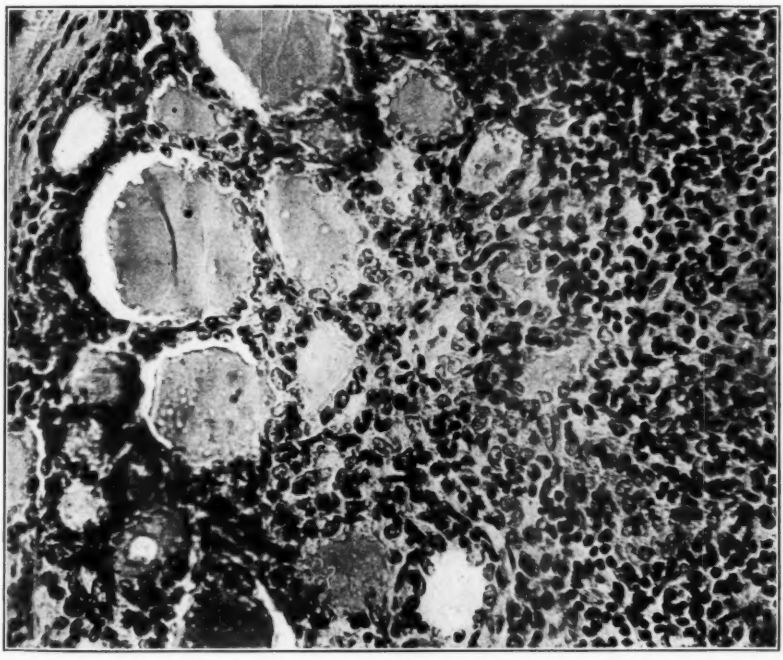


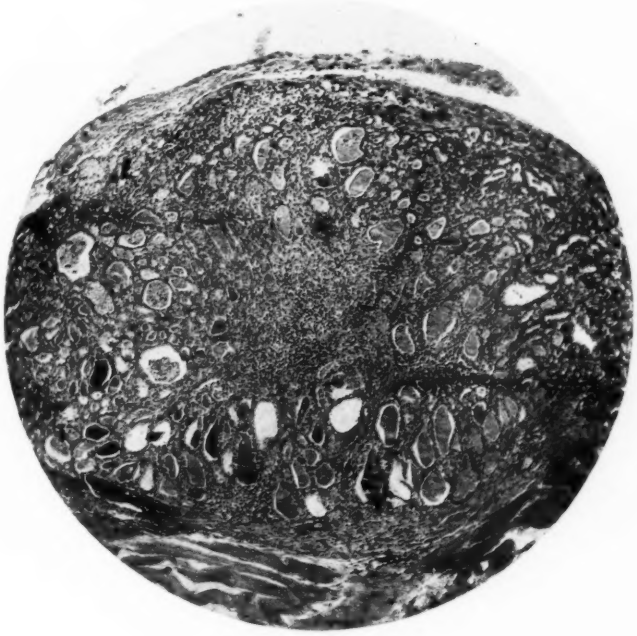
4

Tureen Transplants of Thyroid Glands in Guinea Pigs

PLATE 130

FIG. 5. Experiment VII, Guinea Pig No. 53. Homoiotransplantation to adult animal. This figure shows a degree of preservation of thyroid tissue that is better than that found in the average transplantation into adults after twenty days.

FIG. 6. Higher magnification of Fig. 5.



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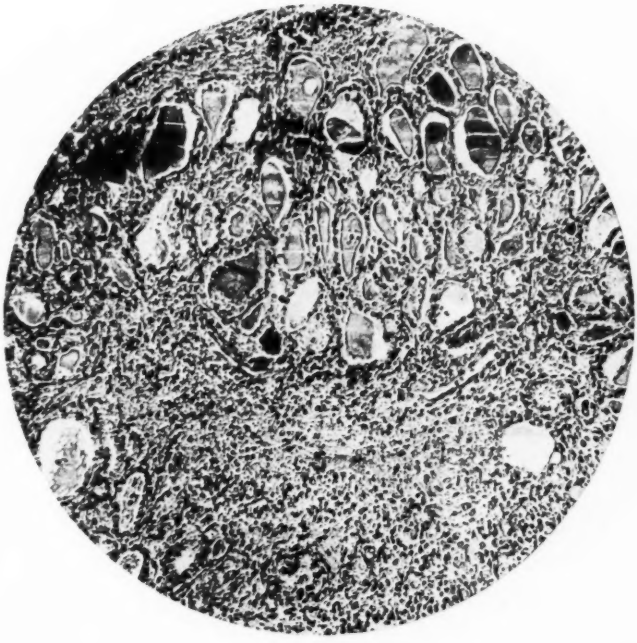


6

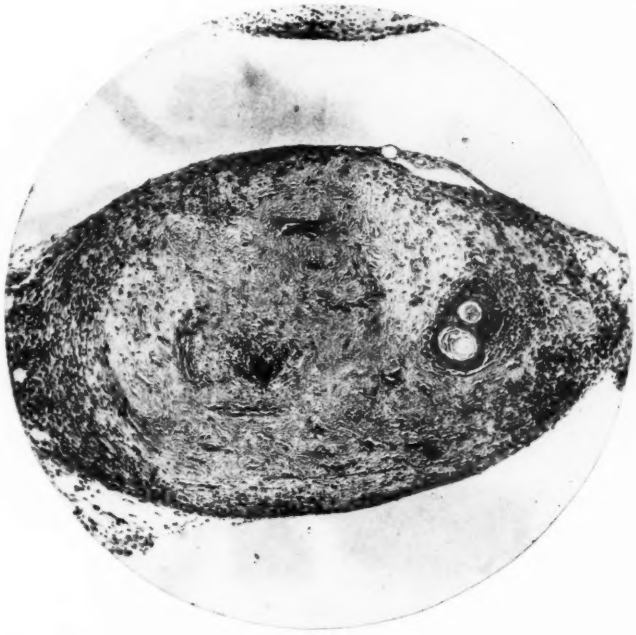
Tureen

Transplants of Thyroid Glands in Guinea Pigs

PLATE 131

FIG. 7. Experiment XLII, Guinea Pig No. 456. Section through thyroid homoiotransplanted to young animal. This photomicrograph shows an unusually severe connective tissue reaction with complete destruction of the transplanted thyroid tissue at the end of twenty days.

FIG. 8. Experiment XXV, Guinea Pig No. 223. Showing peripheral preservation of glandular tissue after twenty days; homoiotransplantation of thyroid into young animal.



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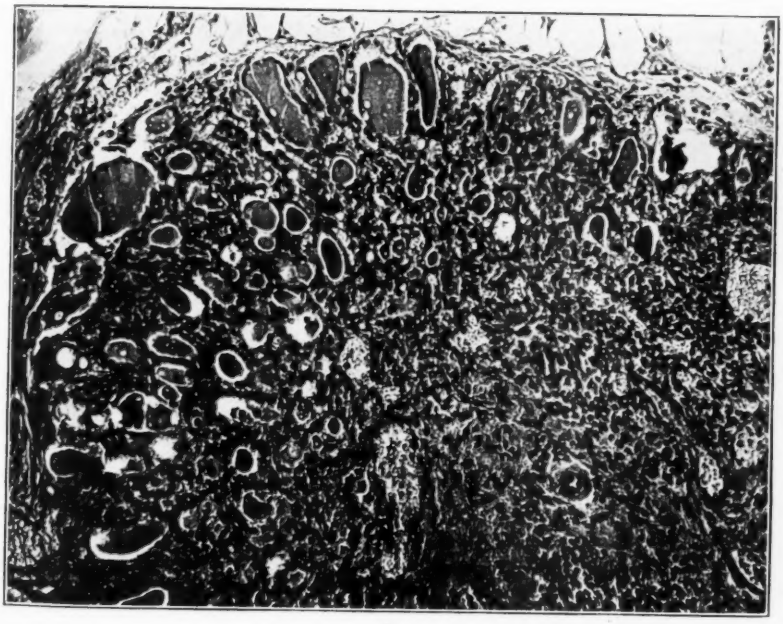


8

Tureen

Transplants of Thyroid Glands in Guinea Pigs

UNIV. OF MICH.

RETICULUM *

F. B. Mallory, M.D., and Frederic Parker, Jr., M.D.

(From the Pathological Laboratory of the Boston City Hospital, Boston, Mass.)

Introduction

In recent years one particular tissue, the so-called reticulo-endothelial system, has been attracting increasing attention both from the physiologic (immunologic) and from the anatomic point of view. Although this tissue has been extensively studied, the resultant views of the different workers in regard to it are by no means in agreement. The two distinctive elements of this system are the endothelial cells and the framework or reticulum of intercellular fibrils which support them. In this paper we are concerned only with the origin and nature of the reticulum. The two undecided questions with regard to it are these: 1. Is reticulum the same as collagen or is it a chemically different substance which may be transformed into collagen? 2. Is reticulum produced by endothelial cells, by so-called reticular cells, or by fibroblasts?

Historical

Under the name of *Gitterfasern*, Kupffer [1] in 1876 described the reticulum occurring in the liver.

Mall [2] in 1896, as the result of digestive experiments and chemical analyses, concluded that there were three kinds of connective tissue fibrils, elastic, collagenous and reticular. He felt that each of these was a distinct variety. He found much reticulum in the capsule and trabeculae of the spleen, but none in the pulp. The exact reverse of these conditions was obtained by later workers who used silver stains.

In 1908 Rössle and Yoshida [3] studied the reticulum of lymph nodes and other organs by means of the Bielschowsky-Maresch silver impregnation method and the Van Gieson stain, and decided that reticulum was closely related to collagen but not identical with it. They found that collagen and reticulum fibrils were often continuous with one another and they felt that the "*Gitterfasernbil-*

* Received for publication April 15, 1927.

dungzellen" were identical with the endothelial cells of the lymph sinuses, but at the same time thought that they might be fibroblasts. They also stated that reticulum was changed to collagen by a metaplasia under certain conditions.

In the same year and by similar methods, Russakoff [4] came to essentially the same conclusions.

Miller [5] in 1923 studied the reticulum content of tubercles in the lung and expressed the opinion that reticulum, while different from collagen, was apparently often changed into the latter.

This hypothesis was also upheld by Foot [6] in a similar study in 1925. Foot's opinion as to the formation of reticulum is that it does not exist as fibrils connected with cells, but is precipitated out in some manner in an intercellular secretion. He as well as several other pathologists apparently regard the reticulo-endothelial cell as the one which forms these fibrils because their diagnoses of the so-called reticulo-endotheliomas are based on the presence and arrangement of these fibrils in certain tumors.

Methods

In view of the differences of opinion in regard to the origin and nature of reticulum, it seemed to us worth while to undertake a comparative study of it by utilizing not only the silver stains but also all the other recognized staining methods for demonstrating intercellular substances.

We made use almost exclusively of tissues which were excised surgically in order to obtain them as fresh as possible. Thin sections of these tissues, not over 2 to 3 mm. thick, were cut and placed in Zenker's fluid within a few minutes after removal from the body and carried through in the usual way. Paraffin sections were employed and the following stains used on each specimen: Foot's modification of the Bielschowsky-Maresch silver impregnation method,[7] Van Gieson's picro-acid fuchsin mixture,[8] and Mallory's eosin or phloxin-methylene blue,[8a] anilin blue collagen,[8b] phosphotungstic acid and phosphomolybdic acid hematoxylin stains.[8c] In addition a limited number of sections were stained using Verocay's technic [9] and the different methods specific for elastic fibrils. Frozen sections of a few tissues fixed in formaldehyde were stained by the original Bielschowsky-Maresch method [9a] and by Perdrau's [10] modification of it.

In our work we chose tumors for examination because the amount of original tissue in them is obviously slight. Furthermore, tumors tend to grow as a single type of cell with only a supporting stroma of blood vessels and connective tissue. In describing our results we shall consider the various types of tumors studied and then the tissues of the reticulo-endothelial system.

With regard to the Bielschowsky silver method, it should be stated that it does not stain fibrin or neuroglia, fibroglia, myoglia or elastic fibrils. Moreover, the results obtained with this method at the edges of the sections where the fixative acted first are not reliable.

Phosphomolybdic acid hematoxylin, like the Verocay method, brings out quite distinctly all the recticulum and collagen in the tissues and has the great advantage of neither causing shrinkage nor freeing paraffin sections from the slide. The best formula for staining collagen seems to be the following:

Water	100 cc.
Phosphomolybdic acid	2 gm.
Hematoxylin	1 gm.

The hematoxylin dissolves readily in the acid. The solution can be ripened for immediate use by the addition of five cc. of a one per cent solution of permanganate of potassium.

Stain paraffin sections of Zenker-fixed tissue for twenty-four hours in the cold, or, if a more intense stain is desired, in the paraffin oven at about 54° C. for two to three hours. Wash in water, dehydrate in ninety-five per cent alcohol followed by absolute, clear in xylol and mount in xylol balsam.

Leiomyoblastoma

Silver stains of a leiomyoma arising from the wall of a vein in the groin showed that each muscle cell, when examined in cross-section, was separated from its neighbors by a thin layer of delicate black-staining fibrils. In other words the muscle cells were completely invested in what is called reticulum. On staining sections, already impregnated with silver, by the anilin blue collagen method it could be seen that the myoglia fibrils were stained red by the acid fuchsin and lay inside of the black reticulum. Where the bundles of muscle cells were running lengthwise, the reticulum was disclosed as delicate wavy fibrils differing morphologically in no respect from the fibrils which form the bundles of ordinary collagen. It was no-

ticeable that the fibrils when occurring singly or in delicate strands stained black, but where they were compacted into bundles, as around blood vessels, they stained only a reddish color.

In a series of leiomyomas of the uterus the same condition was found. Where the layer of intercellular fibrils between the muscle cells was thin and delicate it was stained intensely by the silver method and appeared black. Wherever the muscle cells had died off and disappeared so that the intercellular fibrils were compacted, they no longer stained black but were colored reddish like ordinary collagen. It, therefore, seems reasonable to conclude either that the reticulum was originally collagen or that it had turned into collagen when the muscle cells had disappeared.

Even in the rapidly growing tumors of this type, the leiomyosarcomas, there is the same formation of a reticulum composed of delicate collagen fibrils which must be supplied by the fibroblasts of the stroma. No other cell in the body seems to make such demands for an intercellular substance to surround and support it.

A study of the smooth muscle cells occurring in blood vessels, the gastro-intestinal tract and elsewhere revealed the same peculiarity of staining of the surrounding stroma by the silver method. Where the stroma was in a thin layer it stained black; where it was abundant following the disappearance of the muscle cells, and the formation of patches of sclerosis, it stained light red.

The application of all the other stains for intercellular substances showed that reticulum and collagen stain in exactly the same manner. Morphologically they differ only in the compactness of the fibrils.

The foregoing observations show therefore that smooth muscle cells under all conditions tend to be invested by a thin layer of collagen fibrils which stain black by the silver method. Because of this staining peculiarity and the arrangement around the cells as seen in cross-section, the collagen fibrils are called reticulum. When the reticulum fibrils are compacted owing to the disappearance of muscle cells they stain like collagen fibrils with which morphologically they are identical.

Fibroblastoma

In rapidly growing fibrosarcomas, the collagen produced by the tumor cells is very slight in amount, and is stained black by the silver method. In cross-sections the bundles of cells are seen to be

surrounded by a delicate reticulum, but in longitudinal sections, the reticulum is resolved into delicate wavy fibrils. Stated differently, collagen is stained black by the silver impregnation method when it occurs in single fibrils, in very thin layers or delicate strands.

In more slowly growing fibrosarcomas and in fibromas, the collagen does not stain black, but has a reddish color. In a slowly growing fibromyxosarcoma, on the other hand, the collagen stains intensely black wherever it is separated into individual fibrils or fine strands by the homogeneous intercellular substance.

The tumors of this group, producing their own intercellular fibrils, demonstrate clearly that collagen stains specifically by the silver impregnation method only when it occurs in single separated fibrils or in fine strands or thin layers.

Carcinoma

We chose scirrhous cancers of the breast in order to study the stroma. The fibroblasts are always well developed with numerous fibroglia and collagen fibrils. In addition the elastic fibrils are often abundant in places. The silver preparations showed great numbers of black-staining fibrils around and often between the groups of tumor cells, in the masses of elastic tissue and in the stroma wherever there was edema or infiltration with leucocytes; but no black-staining fibrils were seen where the collagen occurred in coarse bundles or solid masses. Furthermore it was evident everywhere that the black-staining fibrils were always in direct connection with the lightly stained collagen.

With all the other staining methods for intercellular substances, collagen and reticulum reacted and stained in the same way.

Lymphoblastoma

The stroma of a rapidly growing lymphoblastoma consists of a delicate network of intercellular fibrils in the meshes of which lie the tumor cells. The finer threads of this network are stained black by the silver impregnation method, while the coarser are colored black to reddish according to their compactness. The network consists of collagen fibrils arranged in fine or coarse bundles. They are closely applied to the fibroblasts which form a syncytium. The collagen fibrils do not branch, but the bundles of them often seem to branch,

because they course in different directions over the surface of the anastomosing fibroblasts. In the scirrhous type of lymphoblastoma, the reticulum is smaller meshed and often contains many eosinophiles as well as other leucocytes in addition to the tumor cells. In a later stage, as the cells disappear owing to degeneration or emigration, the strands of the reticulum are approximated and stretched more or less definitely in one direction or another. Under these conditions they appear and stain as collagen.

The Reticulo-endothelial System

The information derived from the study of the staining reactions of the intercellular fibrils of certain tumors was applied to the organs of the reticulo-endothelial system.

The stroma of lymph nodes aside from the blood vessels consists of a network of anastomosing fibroblasts which form a syncytium. The collagen produced by the fibroblasts is arranged in a reticulum composed of strands of delicate fibrils which course over the surface of the cells in different directions. Endothelial cells are applied in places to the surface of the reticulum which stains black by the silver method. The lymphocytes are contained in the meshes of the reticulum.

When the axillary lymph nodes are invaded by a scirrhous carcinoma, the stroma cells react as fibroblasts and produce an abundance of collagen.

The capsule and trabeculae of the spleen contain much collagen, many fibroglia and elastic fibrils but no reticulum. The lymph nodules are like those in lymph nodes, and the arteries and veins are similar to the blood vessels elsewhere in the body. The peculiar and characteristic feature of the spleen is the presence of the blood sinuses. They are lined with endothelial cells resting on a stroma of delicate strands of collagen which, on account of its occurrence in this form, stains intensely by the silver method. The fibroblasts which produce the reticulum are characterized by the presence of fibroglia fibrils.

In the organization of infarcts of the spleen, the fibroblasts of the stroma of the pulp produce scar tissue containing much collagen.

Liver

The connective tissue of the liver extends from around the portal and hepatic vessels through the lobule in the form of delicate strands of collagen, which stain black by the silver method. These strands which surround the columns of liver cells lie between them and the endothelial cells lining the sinusoids. They are produced by fibroblasts. In certain types of cirrhosis of the liver, the reticulum of the stroma stains like collagen when it is compacted into patches of sclerosis as the result of degeneration and disappearance of liver cells. When the stroma of the liver is increased in amount as the result of infectious processes (tuberculosis, infectious cirrhosis), collagen is produced in abundance.

In blood vessels, reticulum is present beneath the lining endothelium and between the muscle cells and extends out around the capillaries forming a delicate sheath about them. Reticulum is not formed by the endothelial cells but by the fibroblasts of the stroma for the support of the vessels, in exactly the same manner that it is supplied for the support of smooth muscle cells wherever they occur in the body.

Discussion

The recognition of reticulum depends on two characteristics, structure and staining reaction. The name was originally applied to the fibrillar intercellular substance which forms a network composed of fine strands for the support of cells in various organs and tissues. Later it was discovered that the silver impregnation method, variously modified, stained reticulum black while collagen took on a light brownish or reddish tint according to the method employed. On this account it was generally assumed that the stain was specific, and that reticulum and collagen were chemically different substances. Therefore, the Bielschowsky silver stain in recent years has become the standard means for the recognition of reticulum and has proved a great stimulus to the study of its distribution. It should be noted, however, that reticulum and collagen react alike to all other stains for the demonstration of intercellular substances.

As stated in the introduction to this paper, there are different views in regard to the origin and nature of reticulum. The subject has been confusing, because of the evident close relation between reticulum and collagen, not only chemically but also morphologically, Both are composed of delicate fibrils which are often wavy.

The different views in regard to reticulum will be taken up seriatim.

1. *Is reticulum a precollagenous substance?*

In favor of this view is the early appearance of the reticulum in tubercles and in granulation tissue. In these pathologic conditions the reticulum is in direct continuity with the surrounding collagen into which it changes at a later stage of the process. On the other hand, reticulum persists for a lifetime around smooth muscle cells and yet apparently changes at once to collagen if the cells it surrounds atrophy and disappear as, for example, in leiomyomas. The only change which has happened to the reticulum is a physical one: its fibrils have been brought into close contact with one another; in other words, they have been compacted.

2. *Is reticulum produced by reticular cells?*

This conception would necessitate the recognition of a new type of cell which produces a fibrillar intercellular substance composed of delicate fibrils exactly similar to those produced by the fibroblast, which change to collagen when they are closely packed together.

3. *Is reticulum produced by endothelial cells?*

This view has attained great vogue recently and is strongly advocated by Aschoff [11] in particular. It might be conceivable if one were to limit one's studies to the liver, spleen and lymph nodes; but not if one were to consider all the other organs and tissues of the body. It would mean that the intercellular fibrillar substance in a rapidly growing fibrosarcoma, which is stained black by silver, is produced by the endothelial cells of the blood vessels and not by the fibroblasts of the tumor. In the more slowly growing tumors of this type, however, it is produced entirely by the tumor cells because only collagen is present. It would also indicate that endothelial cells in the stroma of a scirrhous cancer of the breast produced the reticulum around and between the epithelial cells of the tumor at a considerable distance from the blood vessels.

4. *Is reticulum produced by fibroblasts?*

There are several points in support of this view. In a rapidly growing fibrosarcoma, the fibrillar intercellular substance stains like reticulum. In the more slowly growing tumors it stains like collagen and yet the age of the intercellular substance evidently has nothing to do with it. If the fibrils of a slowly growing fibrosarcoma are

separated by fluid or mucin, the collagen fibrils in these parts stain black with silver and must be regarded as reticulum.

In the stroma of scirrhous cancers the fibroblasts produce abundant fibrillar intercellular substance which stains like reticulum or collagen according to whether the fibrils are separated or compacted.

The most important point, however, is this: In its active condition, the fibroblast is characterized by the presence of fibroglia fibrils which run along its surface and which can be demonstrated by means of phosphotungstic acid hematoxylin or the acid fuchsin-anilin blue collagen method. Study of the so-called reticular cells of the spleen, lymph nodes and other organs show that they possess fibroglia fibrils and that they, therefore, are fibroblasts. For this purpose absolutely fresh tissue, just removed from the living body, cut into very thin sections 1 to 2 mm. thick and fixed in Zenker's fluid, must be used.

From this presentation of the results of a comparative study of the fibrillar intercellular substances present in various tumors and organs, it appears to us that reticulum is produced by fibroblasts and is merely collagen occurring as separated fibrils or as delicate strands. Only under this physical condition do the fibrils stain intensely by the silver impregnation method. When, as in leiomyomas, the fibrils of the reticulum are brought into close apposition through degeneration and disappearance of intervening cells, they no longer stain like reticulum but like collagen.

The Bielschowsky silver impregnation stain furnishes an excellent method for the demonstration of the finest fibrils of collagen, but the pictures it presents should be viewed with discretion to avoid misinterpretation. The results obtained by it must be controlled by other, more reliable methods which stain all the collagen present, even if not so intensely.

Summary and Conclusions

All recent work on reticulum has been based on the use of silver stains, chiefly Bielschowsky's and modifications of it. Our results depend on the use of tissue fixed immediately after removal from the living body, and on a comparative study of the various stains for intercellular substances.

Smooth muscle cells in leiomyomas, in the wall of blood vessels and elsewhere are surrounded by delicate fibrils which are stained

black by the silver. When the muscle cells die, the black-staining fibrils around them disappear, but the collagen is increased in amount. The obvious inference is that separated collagen fibrils are stained by silver, but compacted fibrils are not. Myoglia fibrils are not stained by silver. This point can be demonstrated by using the acid fuchsin-anilin blue method on a section already treated with silver.

In fibrosarcomas the collagen is stained black by silver, if it is slight in amount. It is not stained when present in large amounts.

In cancers many loose fibrils surrounding and running between the epithelial cells stain black; the same is true of single fibrils or very small bundles of them embedded in elastic tissue. Stains for fibroglia fibrils following silver stains show that they are not colored by the silver.

The capsule and trabeculae of the spleen contain much collagen, many fibroglia and elastic fibrils but no reticulum. The stroma of the lymph nodules and pulp stains black. Stains for fibroglia fibrils show them to be present running along the surface of the reticular stroma. Hence the cells forming this network are fibroblasts.

It is generally agreed that physically reticulum and collagen are intimately joined together, being always continuous one with the other.

Many believe that reticulum changes to collagen. All reticulum stains by the methods used for collagen, namely, Van Gieson's, Verocay's, anilin blue and phosphomolybdic hematoxylin.

The silver stains are specific for collagen but only under certain physical conditions. Collagen must be separated into individual fibrils, or into very small strands and thin layers by cells, elastic fibrils or fluid.

Reticulum as a chemically distinct intercellular substance does not exist; it is collagen in separated form, rendered prominent by the silver stain.

All collagen is produced by fibroblasts.

There are no reticular cells other than fibroblasts.

Endothelial cells do not produce an intercellular substance.

REFERENCES

1. Kupffer, C. von. *Arch. f. mikr. Anat.*, 1876, xii, 353.
2. Mall, F. P. *Johns Hopkins Hosp. Reports*, 1896, i, 171.
3. Rössle, R., and Yoshida, T. *Beitr. z. path. Anat. u. z. allg. Path.*, 1909, xlv, 110.
4. Russakoff, A. *Beitr. z. path. Anat. u. z. allg. Path.*, 1909, xlv, 476.
5. Miller, W. S. *Am. Rev. Tuberc.* 1923, vii, 141.
6. Foot, N. C. *Am. J. Path.*, 1925, i, 341.
   Foot, N. C., and Day, H. A. *Am. J. Path.*, 1925, i, 431.
7. Foot, N. C. *J. Lab. & Clin. Med.*, 1924, ix, 777.
8. Mallory, F. B., and Wright, J. H. Pathological Technique, Philadelphia & London, 1924, Ed. viii, 119; (8a), 100; (8b), 118; (8c), 149.
9. Schmorl, G. Die Pathologisch-Histologischen Untersuchungsmethoden, Leipzig, 1914, Ed. vii, 155; (9a), 157.
10. Perdrau, J. R. *J. Path. & Bact.*, 1921, xxiv, 117.
11. Aschoff, L. *Beihefte z. med. Klin.*, 1926, xx, 1.

DESCRIPTION OF PLATE

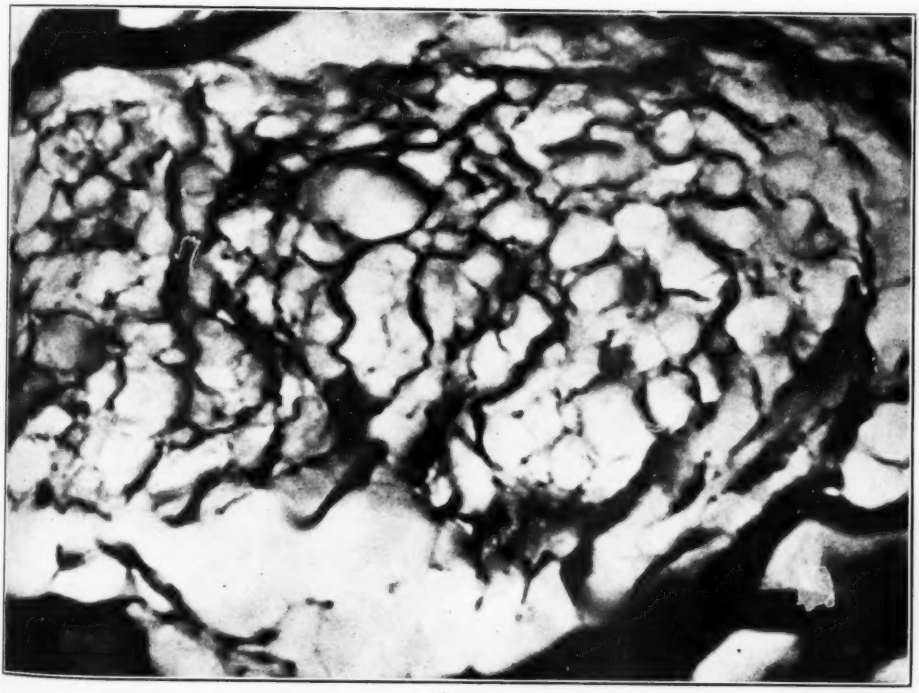
All photomicrographs were made from sections of Zenker-fixed tissue, except Figs. 2 and 3; and stained by Foot's modification of Bielschowsky's silver method, except Figs. 1, 5, 16, 18 and 23.

PLATE 132

FIG. 1. Leiomyoma originating from the wall of a vein in the inguinal region. Stained with phosphotungstic acid hematoxylin to show the myoglia fibrils. × 500.

FIG. 2. Cross-section of bundle of smooth muscle cells from the same tumor to demonstrate reticulum surrounding them. Formaldehyde fixation, Foot's silver method. × 1000.

1



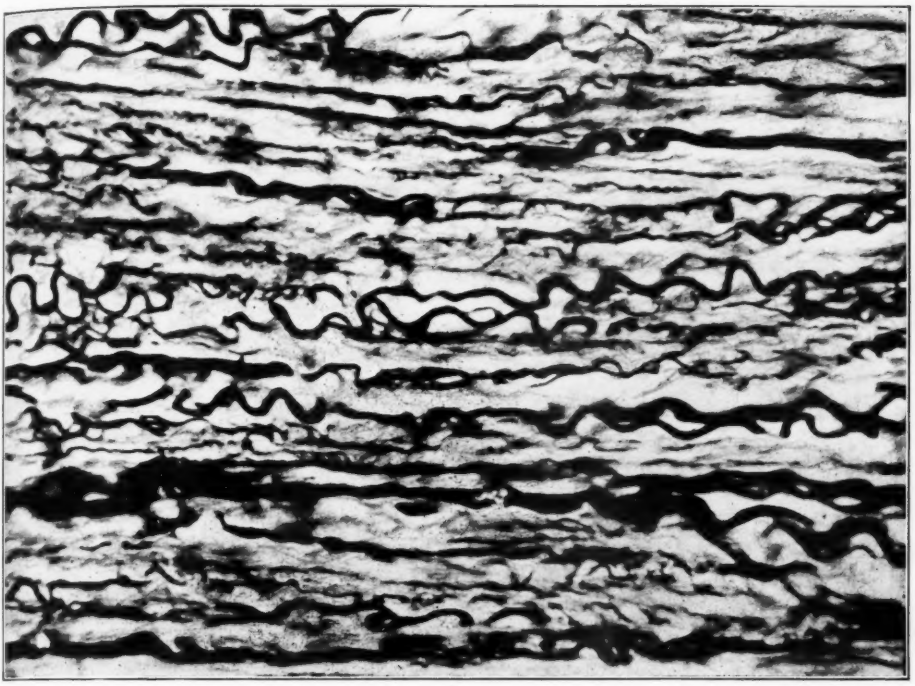
2

Mallory and Parker Reticulum

PLATE 133

FIG. 3. Longitudinal section of same leiomyoma (Figs. 1 and 2) to show reticulum resolved into wavy collagen fibrils occurring in single and in fine strands. Fixative and staining same as Fig. 2. × 1000.

FIG. 4. Leiomyoma of uterus. Reticulum around muscle fibrils. Where the cells have degenerated and disappeared the reticulum fibrils have been compacted into collagen and stain very slightly. × 1000.



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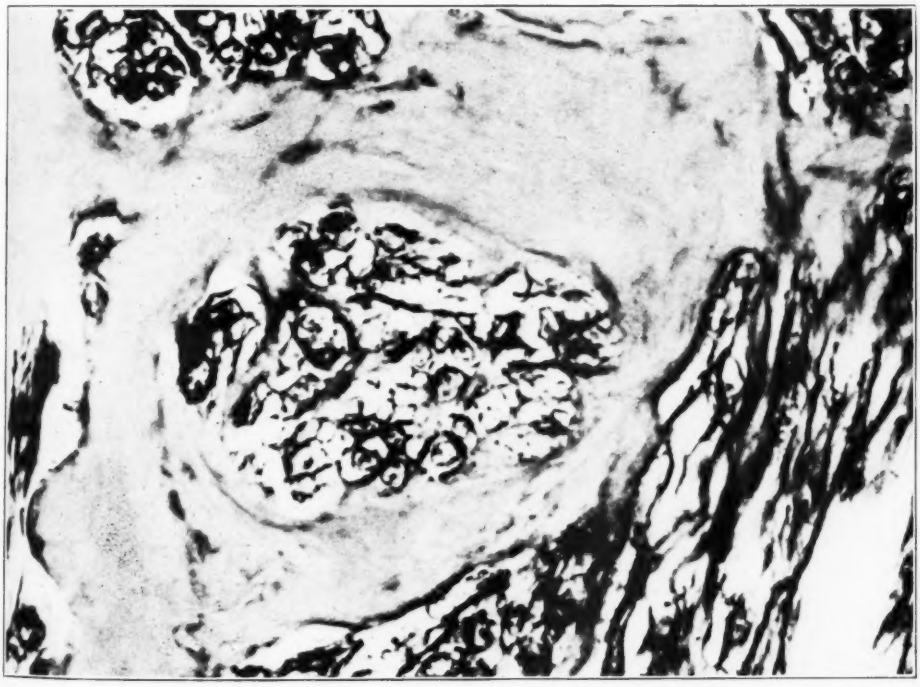

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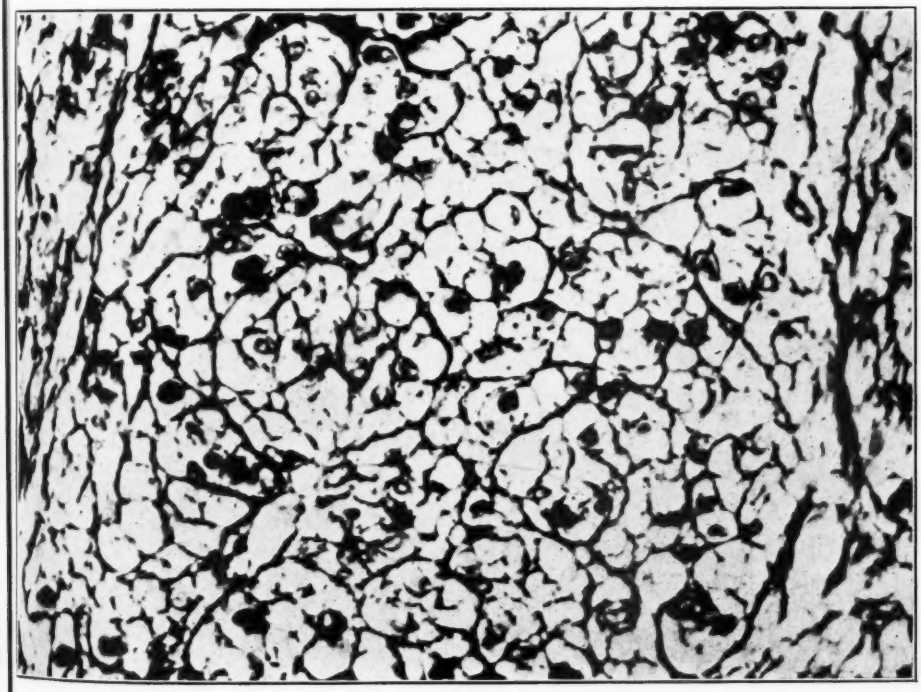
Mallory and Parker Reticulum

PLATE 134

FIG. 5. From a metastasis in a cervical vertebra of a leiomyosarcoma of the uterus. Stained with phosphotungstic acid hematoxylin to show the myoglia fibrils. One diaster present. × 1000.

FIG. 6. From a metastasis of the same leiomyosarcoma to the orbit to show the reticulum between the cells. × 1000.

5



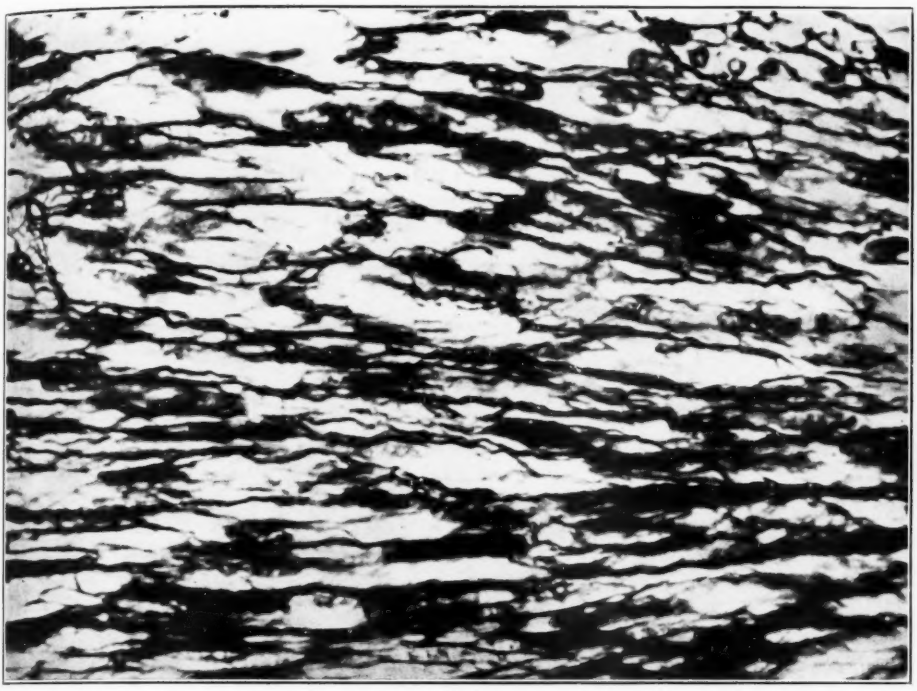
6

Mallory and Parker Reticulum

PLATE 135

FIG. 7. Longitudinal section of the metastasis (Fig. 5). The reticulum is seen here resolved into collagen fibrils. × 1000.

FIG. 8. A rapidly growing rhabdomyosarcoma projecting into a lymphatic. The fibroblasts of the stroma have furnished a reticulum surrounding single tumor cells or small groups of them. × 250.



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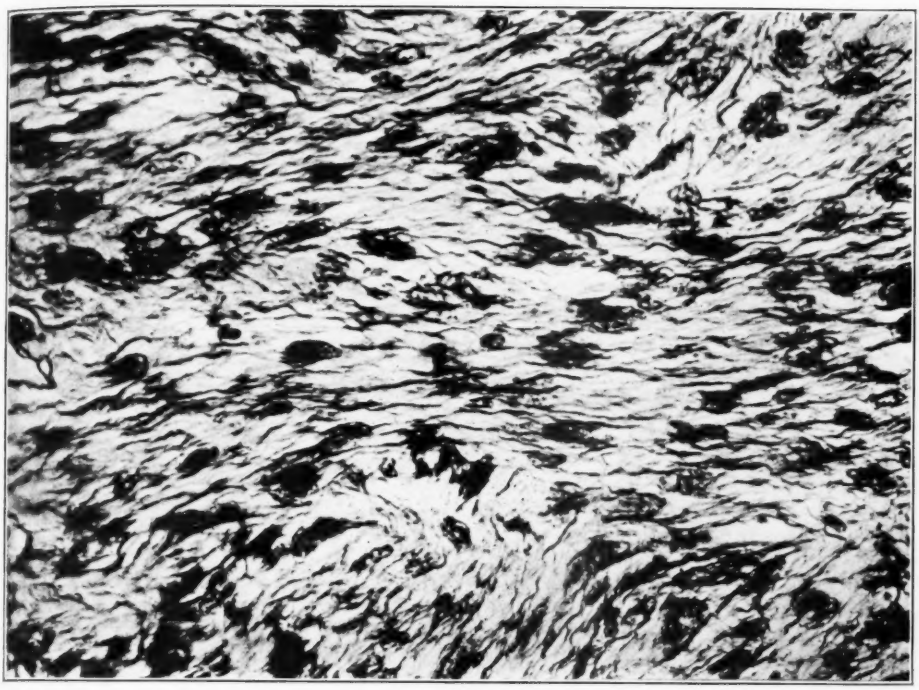
8

Mallory and Parker Reticulum

Plate 136

Fig. 9. A rapidly growing fibrosarcoma of the kidney. The fibrillar intercellular substance is stained black by the silver method. One mitosis in the center of the field. × 500.

Fig. 10. Fibrosarcoma of the pectoral muscle in a child. The fibrillar intercellular substance is stained black by the silver method. × 500.



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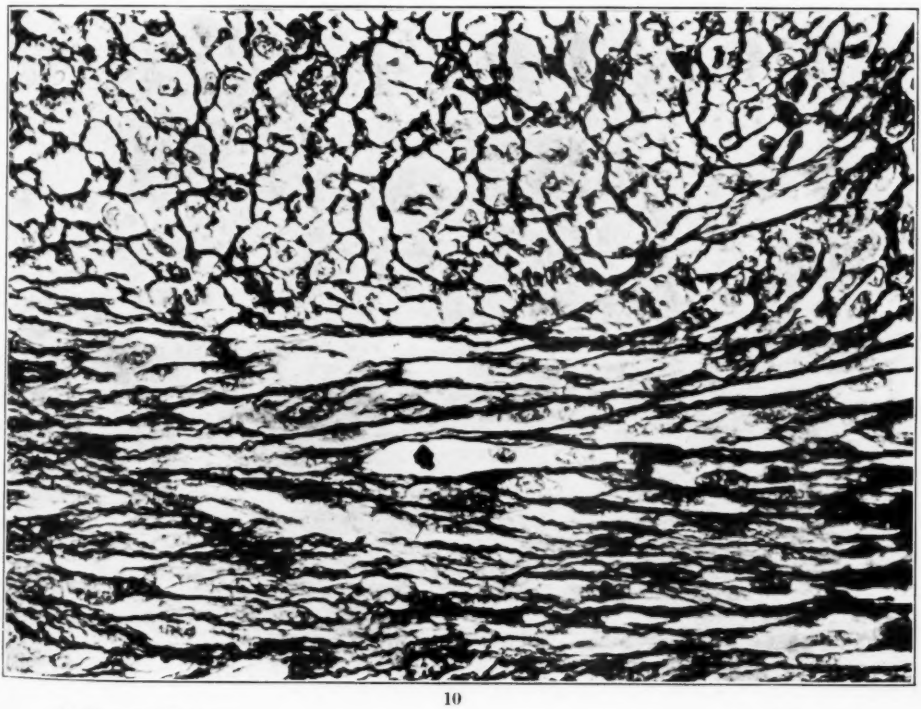


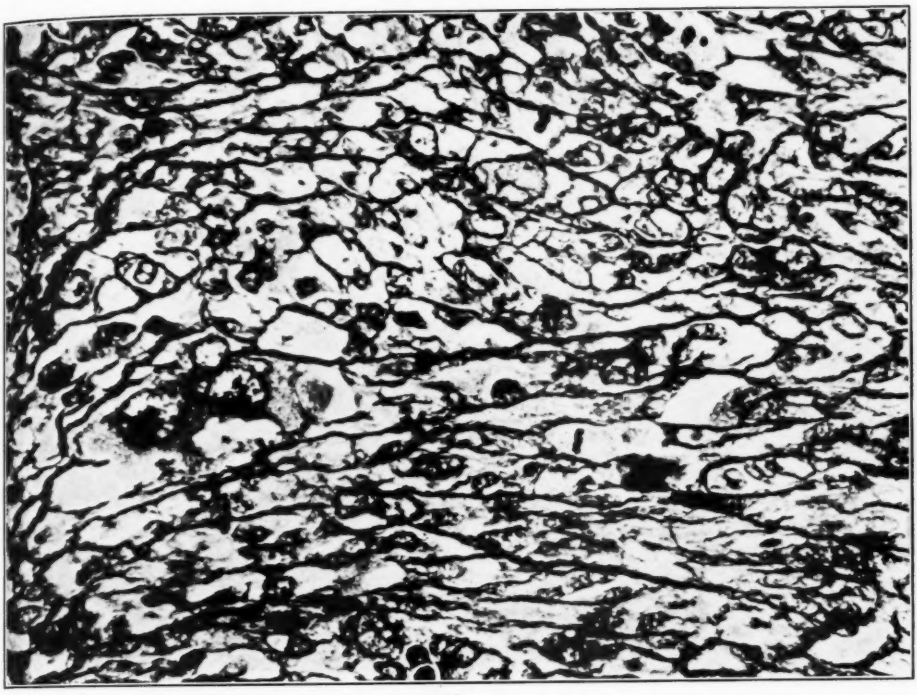
10

Mallory and Parker Reticulum

PLATE 137

FIG. 11. Same tumor (Fig. 10) showing mitoses and one tumor giant cell. × 500.

FIG. 12. A fibrosarcoma in which the fibrils run in all directions. All the finer ones stain black by the silver method, the coarser ones less intensely. The wavy fibrils in the center are in the wall of a dilated capillary. × 1000.



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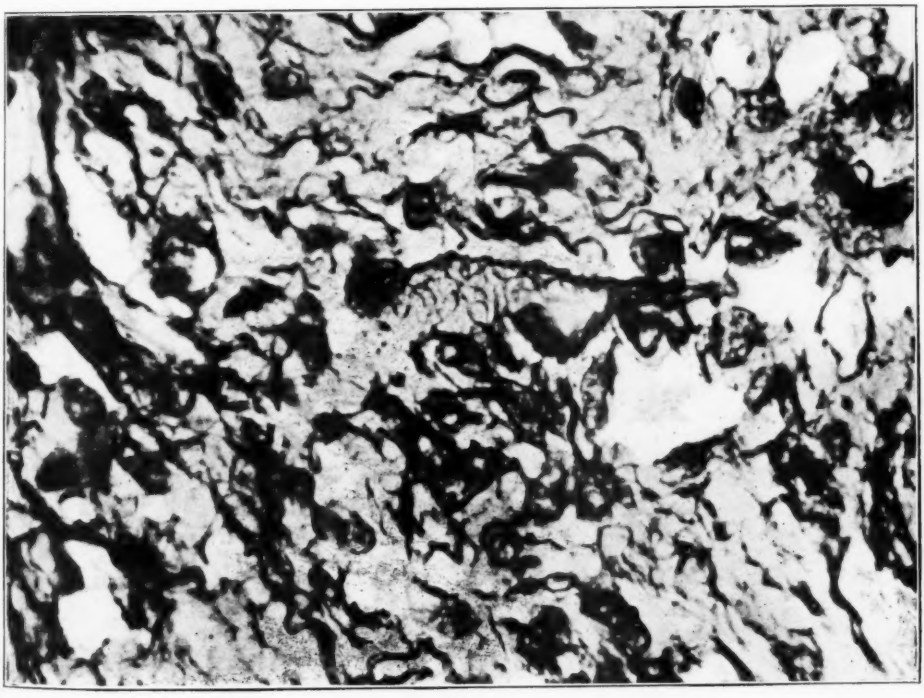

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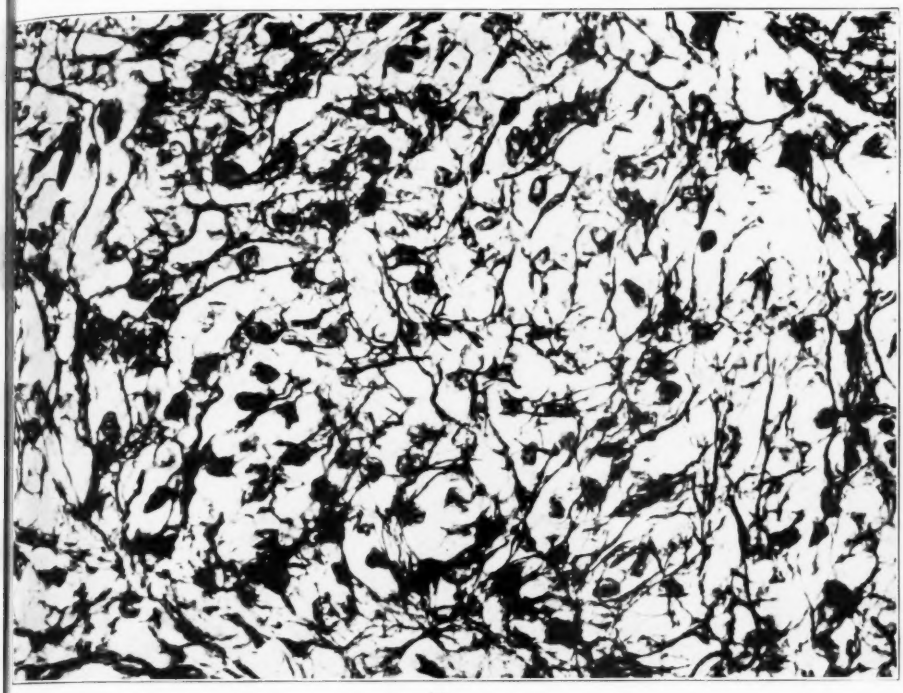
Mallory and Parker Reticulum

PLATE 138

FIG. 13. An edematous fibrosarcoma of the uterus. The separated fibrils all stain deep black by the silver method. × 500.

FIG. 14. Fibromyxosarcoma. The fibrils in the myxomatous portion stain black. × 500.



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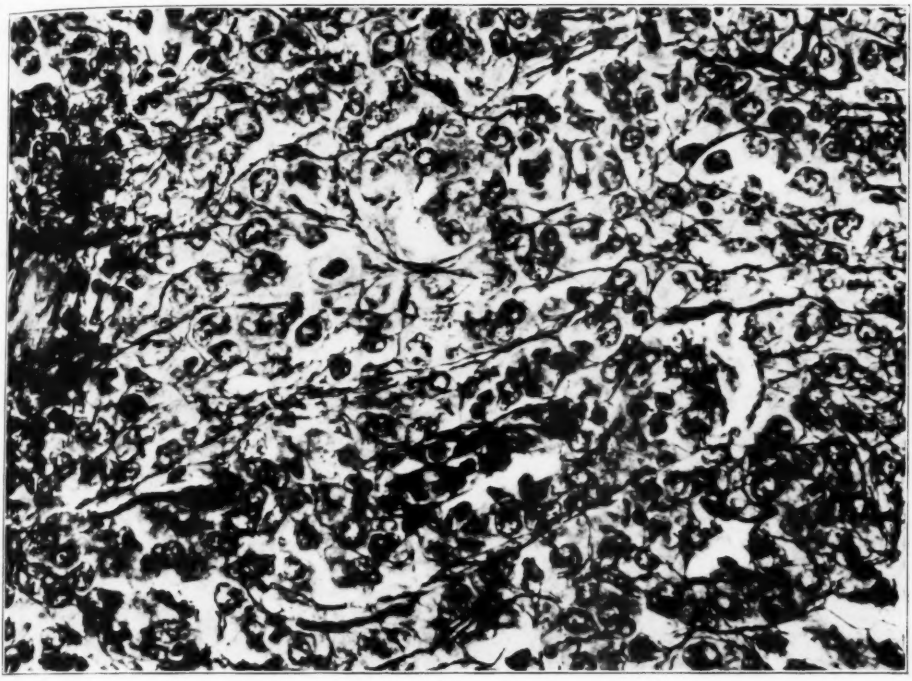
14

Mallory and Parker Reticulum

PLATE 139

FIG. 15. Lymphoblastoma. One mitosis present. The reticulum furnished by the fibroblasts of the stroma is stained black. × 500.

FIG. 16. A phosphotungstic acid hematoxylin stain of the same tumor to demonstrate the fibroglia fibrils of the fibroblasts. × 1000.



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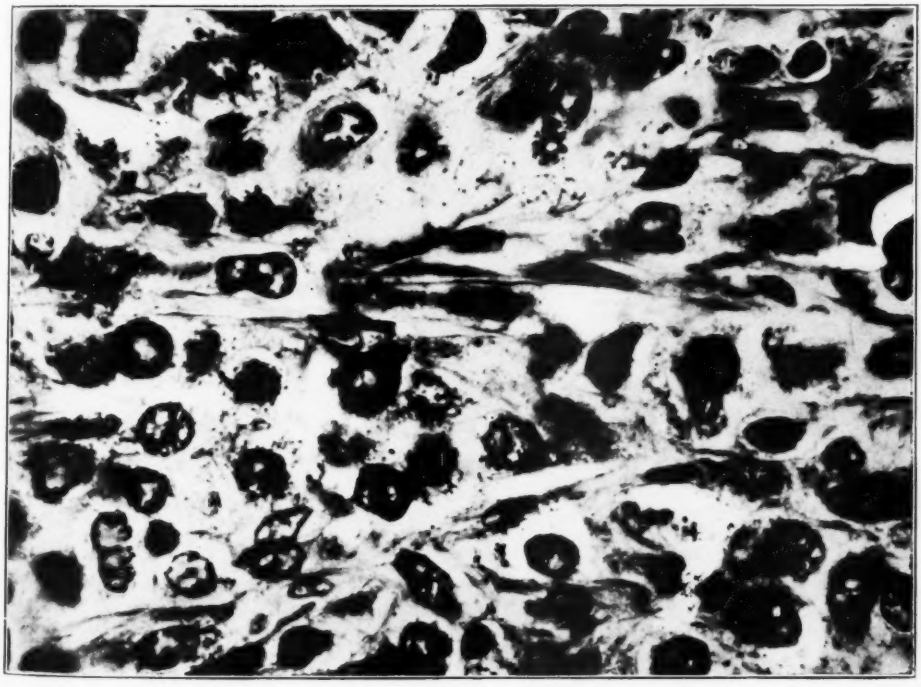


16

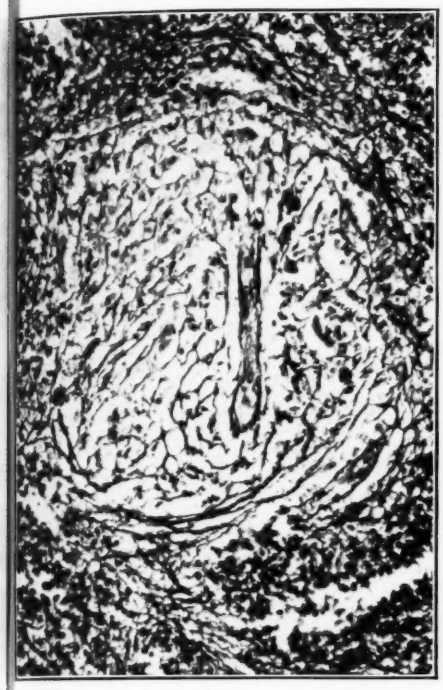
Mallory and Parker Reticulum

PLATE 140

FIG. 17. A silver stain of the reticulum in a lymph nodule of the spleen. × 250.

FIG. 18. Another lymph nodule in the same spleen stained by phosphomolybdic acid hematoxylin. × 250.

FIG. 19. Cancer of the breast. The collagen fibrils running near and between the tumor cells are stained intensely black. × 500.



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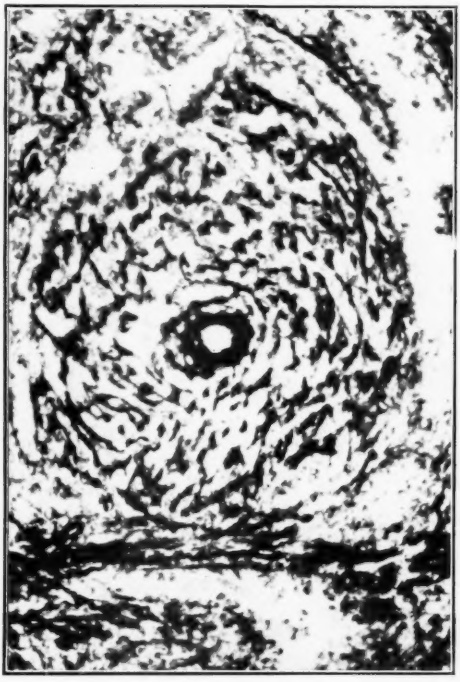

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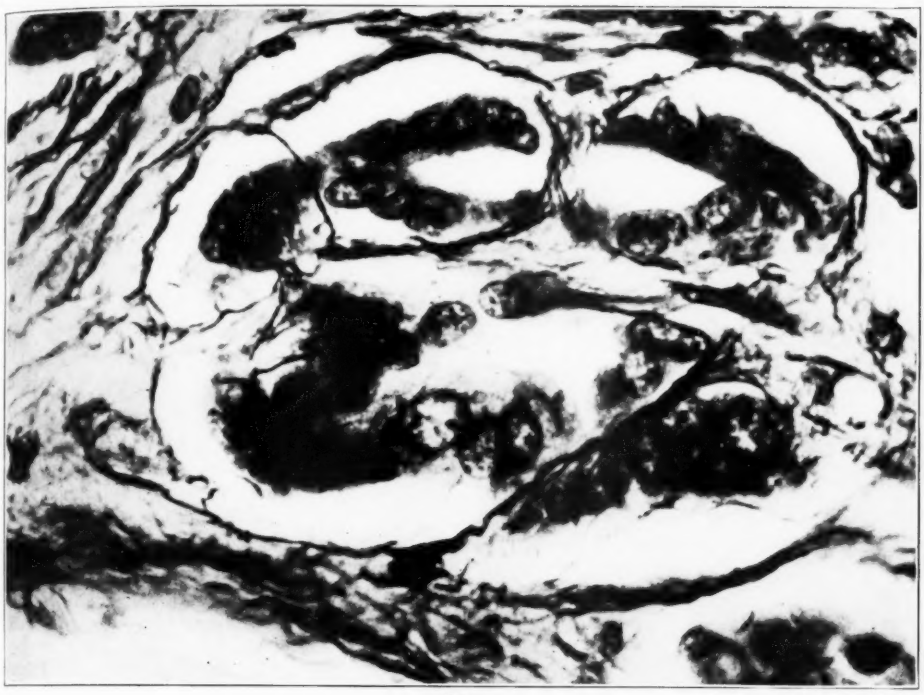
Mallory and Parker Reticulum

PLATE 141

FIG. 20. A similar condition is shown in another illustration from the same tumor (Fig. 19). × 1000.

FIG. 21. In the same cancer the collagen fibrils, separated by elastic fibrils, stain intensely. × 500.



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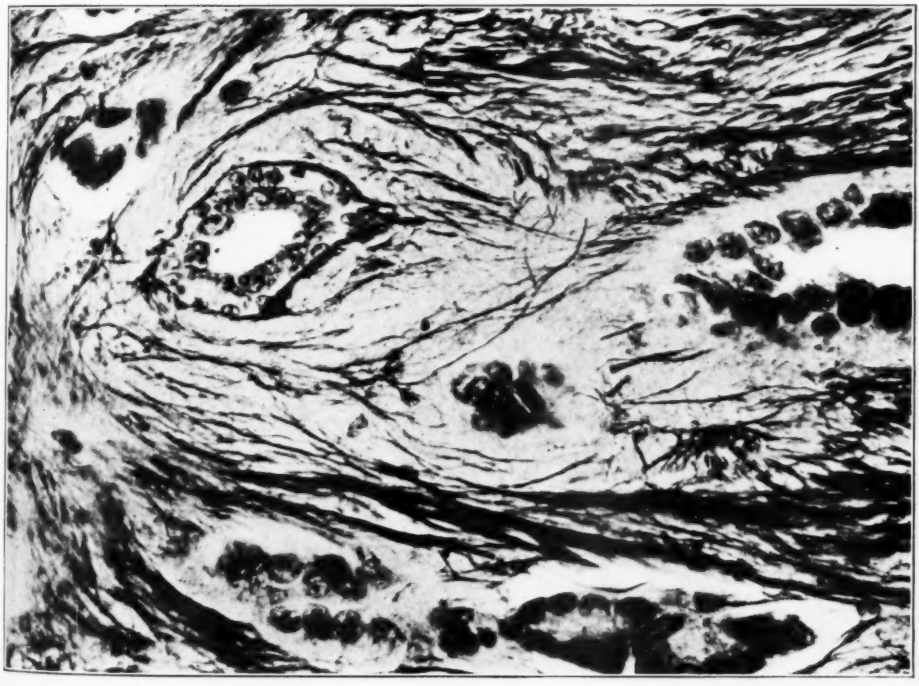

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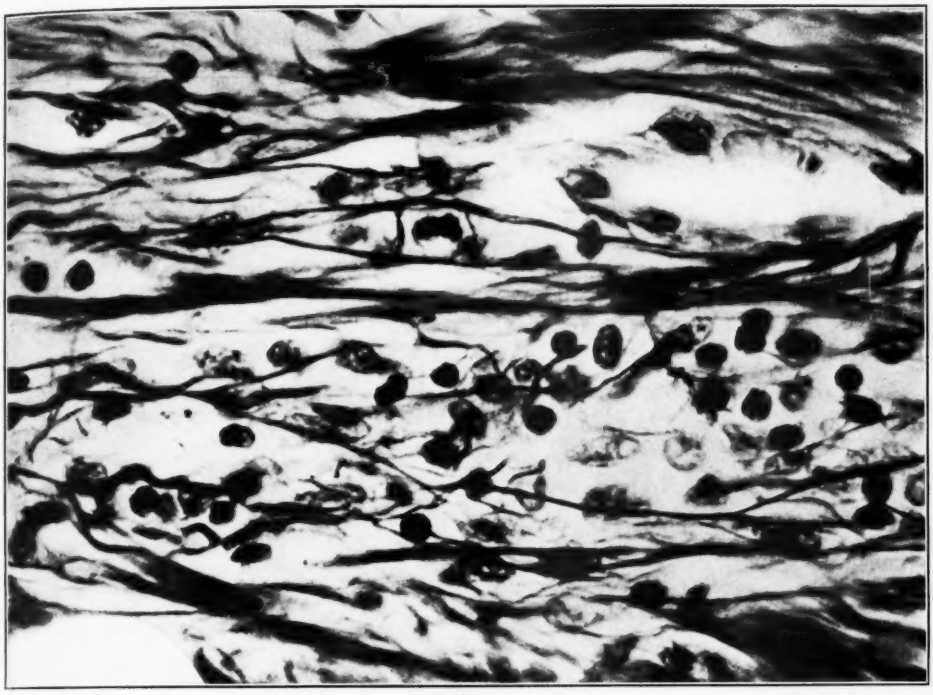
Mallory and Parker Reticulum

PLATE 142

FIG. 22. The same holds true of fibrils separated by an infiltration of lymphocytes. × 1000.

FIG. 23. Cancer of the breast stained by phosphomolybdic acid hematoxylin. All the collagen fibrils, both those that are separated and those that are compacted, stain deeply. × 250.



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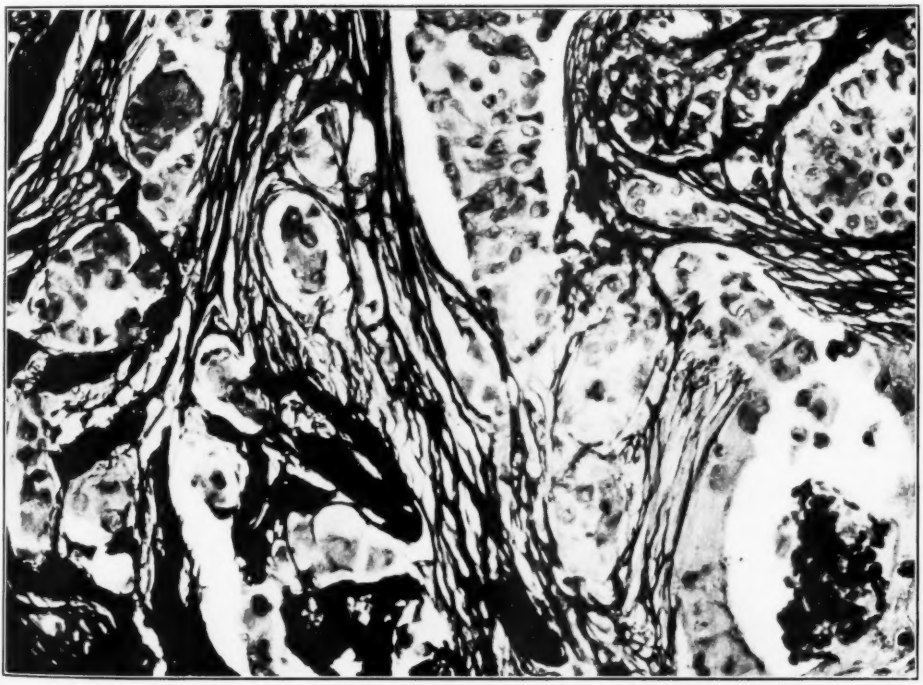

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Mallory and Parker Reticulum

UNIV. OF MICH.

THE INVOLVEMENT OF THE AORTIC VALVE IN SYPHILITIC AORTITIS *

O. SAPHIR, M.D. AND R. W. SCOTT, M.D.

(From the Departments of Pathology and Medicine of the Cleveland City Hospital and School of Medicine, Western Reserve University, Cleveland, O.)

INTRODUCTION

Studies of syphilitic vascular disease have been numerous. The wealth of material at our disposal has made it seem wise to study the alterations which occur in the aortic valve leaflets in relation to the changes in the aorta.

Most text-books state that syphilis may involve the aortic valve from the aorta through the commissure, and explain the insufficiency as due to shortening and thickening of the valve leaflets together with a widened aortic ring which intensifies the incompetence. This is the view of MacCallum,[16] who, however, admits that early stages have not been studied.

LITERATURE

Heller [10] and Chiari [7] in their classical description of luetic aortitis state that the syphilitic process may involve the aortic valve, causing insufficiency; and may involve the mouths of the coronaries, resulting in partial or complete occlusion. They, however, give no detailed description of the valve. Fabris [9] states that syphilis of the valve starts in the region of the commissure. He pictures the lesions there as being fibrous and productive, but with no tendency to either fatty change, atheromatous change or calcification. He thinks that hyaline plaques at the site of the valve attachments may spread the commissures, causing insufficiency. Engel [8] in a subsequent article expresses the same views as Fabris, but neither gives any details concerning the histology nor pathogenesis of the lesion.

Koch [13] describes a severe case of syphilitic incompetence of the aortic valve. Three cusps were present, but the left leaflet was almost completely adherent to the aortic intima. Histologic examination revealed diffuse fibrosis of the cusp and fibrosis of the upper part of the intima of the aorta.

* Received for publication June 7, 1927.

Lupu [15] from an examination of fifteen cases of syphilitic aortic insufficiency concludes that the changes of the aortic valve in syphilis are due to a productive inflammation, and that the process spreads from the aorta through the commissure into the free margins of the leaflets, producing a marked thickening. He attributes the insufficiency to a shrinkage of the thickened margins of the cusps and shortening of their horizontal diameter.

Doehle [5] states that syphilis of the aortic valve produces a thickening of the commissures and of the leaflets, which may be spread by the formation of a large hyaline plaque. He holds that the histologic picture shows no changes characteristic of syphilis.

Miloslavich [17] explains the insufficiency of the aortic valve by thickened, dense and shrunken aortic leaflets.

Cabot [6] mentions that syphilitic valve lesions are characterized grossly by a spreading of the commissure.

Karsner [12] states that the syphilitic process of the valve begins at the junctions of the leaflets, producing adhesions to the neighboring sinus walls and widening of the commissures.

This short survey shows that there is a divergence of opinion as to the nature of the involvement of the aortic valve in syphilis.

Material

Our study is based on necropsy findings of seventy-one cases. Blocks were made from various parts of the ascending and descending aorta and from different parts of the aortic valve. They were fixed in 10 per cent formalin and embedded in paraffin. The sections were cut 5 to 8 microns thick, and most of them stained with hematoxylin and eosin; others stained with Weigert's elastic tissue and Van Gieson's connective tissue stains. In three cases serial sections were made of the entire valve. Sections of the aortic leaflets and aortic wall of the sinus of Valsalva of ten cases of chronic nonsyphilitic endocarditis of the aortic valve were used as controls, and in addition serial sections of a normal aortic valve.

Gross Findings

All cases show fusion between the lateral portion of the leaflets of the aortic valve and the aortic wall of the sinus of Valsalva. Normally the aortic leaflets are attached to one point of the aorta called the commissure. Fusion between the lateral part of the cusps and

the aorta naturally leads to a widening or separation of the commissures. Sixty cases show hyaline plaques of varying size in the region of the commissures. No relation could be established between the size of these plaques and the degree of separation of the commissures. The central portion of the free margin of the cusps in a vast majority of cases shows a marked thickening; eversion or rolling in twenty cases, and retraction in five cases only. In twenty-five instances the mouths of the coronaries are constricted. Twelve times both vessels are encroached upon; seven times the right and six times the left. In three cases the coronary openings are displaced.

In the majority of cases the most marked intimal changes of the aorta are found in a segment 4 cm. in length immediately above the aortic valve. Intimal thickening, marked hyalinization and sometimes calcification are present, and in addition, varying numbers of depressed scars and longitudinal wrinkles. In eight cases these changes are present only in the ascending aorta. The remainder of the cases show wider extensions with a distinct decrease in the severity of the changes in the arch and in the descending aorta. In a few cases, small atheromatous ulcers are observed. One case, a colored female 22 years of age who died of lobar pneumonia, shows only very few reddish, depressed, longitudinal lines above the commissures and no noteworthy intimal changes. This case is considered the earliest in our collection. The aortic valve shows marked adhesions between the leaflets and the aortic intima, in addition to the above-mentioned changes. The lesion was clinically silent. The ascending and descending portions of the aorta of our cases show, besides intimal changes, the wrinkles and puckerings of syphilis, which are found more frequently in the ascending aorta and in the arch.

The hearts vary in weight from 300 gm. to 1000 gm., most of them being approximately 500 gm. The ages range from 22 years up to 72 years. The majority of patients are about 40 years of age. In twenty-one cases the interval between the primary lesions of syphilis and the first clinical signs of aortic insufficiency could be established. The most common interval is about 20 years.

Histologic Findings

Histologically, perivascular infiltrations of round cells are found in all sections, but more marked in the adventitia. The vasa vasorum of the adventitia, particularly in the early cases, show marked

proliferation of endothelial cells leading to a partial or even complete obliteration of their lumina. Some vessels are hardly recognized without the aid of an elastic tissue stain; others are identified by the presence of a few red blood cells in the lumen. The elastic fibers are often found split and increased in number. In spite of the elastic stain it is often impossible to determine whether the endothelial cells obliterating the lumen are intimal or adventitial in origin, a fact noted by Jakob [11] in his study of endarteritis obliterans of the brain vessels. Degenerative changes, however, of these proliferated cells are not observed. Since sections were taken from different parts of the aorta, it could be demonstrated that there are relatively more vasa vasorum and more pronounced obliterative changes nearer to the root of the aorta. This is striking in early cases, especially in the region of the commissure. In a number of sections taken from early cases, vessel changes are seen unaccompanied by round cell infiltrations. Old cases, however, show a marked new formation of connective tissue throughout the adventitia.

The media also shows perivascular round cell infiltrations about vasa vasorum. Here vessel changes are less frequent. The most prominent features, however, are broken elastic fibers and necrotic areas independent of lymphocytic infiltration. Early cases, which were the seat of a slight obliterative endarteritis of the adventitial vessels, show barely demonstrable lesions of the media.

In our early cases the inner portions of the media and the adjacent intima in those areas corresponding grossly to the commissures show changes very suggestive of mucoid degeneration. Numerous stellate cells are present, often characteristically triangular in shape with processes projecting in three directions. These cells are separated by a light bluish stained homogeneous intercellular material which gives a positive mucoid reaction (Bismark brown). No normal intima is seen in this region. Similar changes are found about the openings of the coronary arteries and the lateral portions of the cusps of the valves. The intima in other parts shows a slight increase in connective tissue and a few areas of hyalinization. There is a new formation of small sized vessels around the areas which show most marked degenerative changes. A slight infiltration of endothelial cells, lymphocytes and a few leucocytes, is found throughout, but chiefly located around the zones of mucoid degeneration.

Early cases show only the mucoid degeneration and a few cellular elements, but no hyalinization.

The adherent parts of the leaflets show a new formation of small-sized vessels extending from the intima of the aorta through the commissures. Rather numerous fibroblasts and endothelial cells, some of which are spread by an edema-like material, are present in these parts, together with a few areas of hyalinization. The central portions of the cusps of early cases, which are not thickened grossly, show, in contrast to the lateral portion, no changes histologically.

Apparently in the older lesions the degenerative changes are less pronounced and chronic inflammation more evident. The lateral non-adherent portions of the leaflets of the valves in the older lesions show many new connective tissue cells separated by an edema-like, homogeneous material containing a few lymphocytes, plasma cells and endothelial cells. In examining the serial sections, it is evident that hyalinization and chronic inflammatory processes are more marked the closer to the adherent portion of the cusps the sections are taken. In the region of the commissure new vessels extend from the aorta into the adjacent parts of the valve. The largest amount of hyalin and the fewest number of cellular elements are observed here. The central portion of the valve leaflets, in cases which are grossly thickened, show only fibrosis, very few cells, but no vessels. The areas around the mouths of the coronaries in some cases show fibrosis associated with endothelial cells, plasma cells, lymphocytes and areas of hyalin. We could, however, always demonstrate a few areas of degenerative changes mentioned previously.

Control

Our cases show grossly either adhesions between the leaflets (stenosis) or retraction of the cusps (insufficiency). None shows separation of commissures or adhesions between the cusps and aortic wall of the sinus of Valsalva. Six of ten cases show arteriosclerotic lesions in the ascending aorta, but in three instances the lesions are slight. The arteriosclerotic lesions consist of yellow areas of intimal thickening, areas of calcification and atheromatous ulcers. None of the cases shows any signs of syphilis.

Microscopically, numerous fibroblasts and a few endothelial cells are present in the valve leaflets. A new formation of vessels is observed in some cases. It could, however, be demonstrated that these

vessels extend down to the base of the valve. This observation supports Aschoff's finding, namely that these vessels are branches of the vessels normally found in the base of the valve. No extension of vessels through the commissures is observed. Hyalinization and calcification are found frequently. The region of the commissure and the coronary openings show nothing unusual save for a varying amount of intimal thickening.

Discussion

Our cases of syphilis show uniformly the widening of the commissures, which is pathognomonic of syphilis of the aortic valve. The common opinion is that the commissures are spread by the formation of hyaline plaques at the site of attachment of the leaflets to the aorta, extending wedge-shaped between the commissures. The mere fact that some of our cases show widening of the commissures without the formation of any hyalinized area in this region leads us to believe that there must be another explanation for the separation of the commissures.

The most constant histologic findings are endarteritis obliterans of the vasa vasorum and perivascular infiltration of lymphocytes in the adventitia. We have stated that our early cases show the endarteritis in a certain number of specimens without perivascular infiltration. Backhaus,[2] Warthin[19, 20] and lately Waite [18] hold that obliterative endarteritis of the vasa vasorum of the aortic adventitia is primary, a finding with which, in general, we agree. The perivascular infiltration also is found more frequently and more pronounced in the adventitia, as already mentioned by Larkin and Levi.[14] We must assume that the endarteritis occurs in vessels of a certain caliber only, because the changes occur almost entirely in the adventitial vessels.

The media shows numerous necrotic areas independent of lymphocytic infiltration. For this reason we do not believe that these necrotic areas are always the result of a breaking down of the lymphocytic infiltrations. We are of the opinion that necrosis is a sequence of obliterative endarteritis of the vasa vasorum.

In the intima and inner parts of the media, in the region of the commissures, the mucoid degeneration is found. Warthin states that he was able to demonstrate spirochetes in hearts in areas which microscopically are mucoid in character. Chiari mentions in his

tables a myxomatous degeneration in the aorta, but gives no details. Very recently Brown and Pearce [3] found myxomatous changes in the myocardium of syphilitic rabbit hearts. It must be assumed that the mucoid degeneration of the media and intima is primarily due to nutritional disturbances. While the media is supplied by the vasa vasorum alone, the intima and inner parts of the media are supplied by the circulating blood also. The latter prevents necrosis but is apparently insufficient to prevent degenerative changes. The degenerated areas become organized, as indicated by a new formation of vessels, endothelial cells, fibroblasts and young connective tissue. The perivascular infiltration of the newly formed vessels shows that the process does not undergo healing, but proceeds continually as chronic inflammation. In older cases, the number of cells in the newly formed connective tissue decreases and hyalinization occurs. The constant presence of the newly formed vessels prevents ulceration of these areas. The proliferative intimal changes with the formation of large hyaline plaques without ulceration are, in our opinion, directly referable to the syphilitic process of the adventitia and, as a rule, not to a coincidental arteriosclerosis. While arteriosclerosis often leads to ulcer formation in the intima, syphilis produces a productive inflammation without ulcer formation.

The most extensive lesions of the aorta are found in a segment of about 4 cm. above the aortic value. As Spalteholz states, these areas show more vasa vasorum than any other region of the aorta. Syphilis is a primary disease of the vasa vasorum and therefore of greater significance in areas containing a large number of vasa vasorum. It appears in the vast majority of cases that syphilitic valve lesions occur only with involvement of this segment of the aorta.

The following hypothesis appears best to explain the separation of the commissure: The lateral portion of the aortic valve leaflets is, according to Bayne-Jones,[4] supplied by the vasa vasorum from the aorta. The central parts of the free margins do not show vascularization. In the event of a nutritional disturbance only those areas supplied by the vessels in question will show degenerative changes. These are soon followed by a chronic inflammatory reaction. Organization of the inflammatory exudate of the affected parts of the leaflets and the corresponding area of the aortic intima of the sinus of Valsalva finally produces adhesions between these

two regions which later may become hyalinized. The central portion of the cusps is not supplied by the vasa vasorum and therefore shows no characteristic syphilitic lesions.

The free margins of the central portions of the cusps are, in some cases, rolled and markedly thickened. No changes similar to those of the lateral portion are demonstrable. We believe that these areas of thickening are caused by the continuous mechanical pressure of the regurgitating blood associated with the insufficiency of the valve. The margins of the cusps in early cases are not thickened.

The changes around the mouths of the coronary arteries, also primarily degenerative in character, likewise undergo a chronic inflammatory reaction resulting in a new formation of connective tissue with hyalinization, with possible stenosis, or displacement of the coronary orifice.

Conclusions

1. The earliest lesion of syphilis of the aorta is an obliterative endarteritis of the vasa vasorum. The term "mesaortitis syphilitica" therefore gives an erroneous conception of the disease and "aortitis syphilitica" seems more appropriate.

2. Necrosis of the media is probably secondary and attributable to nutritional disturbances.

3. The changes of the aortic valve due to syphilis are characterized grossly by adhesions between the lateral parts of the leaflets and the aortic intima, leading to a separation of the commissures. The formation of hyaline plaques in this area is secondary.

4. Histologically the affected parts of the leaflets and the aortic wall of the sinus of Valsalva show first degenerative and later chronic inflammatory changes.

We are indebted to Professor H. T. Karsner for his helpful coöperation in this study.

REFERENCES

1. Aschoff, L. Pathologische Anatomie, Jena, 1919.
2. Backhaus, F. B. Ueber Mesarteritis syphilitica und deren Beziehung zur Aneurysmenbildung der Aorta. *Beitr. z. path. Anat. u. z. Allg. Path.*, 1897, xxii, 417.
3. Brown, W. H., and Pearce, L. Syphilitic myocarditis in the rabbit. *J. Exper. Med.*, 1926, xliii, 501.
4. Bayne-Jones, S. The blood vessels of the heart valves. *Am. J. Anat.*, 1917, xxi, 499.

5. Doehle. Ueber das Charakteristische der syphilitischen Erkrankung der Aortenklappen. *München. med. Wchnschr.*, 1921, lxviii, 1000.
6. Cabot, R. Facts on the heart, Philadelphia, 1926.
7. Chiari, H. Ueber die syphilitischen Aortenerkrankungen. *Verhandl. d. deutsch. path. Gesellsch.*, 1904, xv, 137.
8. Engel, F. Über die Veraenderungen an den Aorten Klappen bei Aorten Syphilis. Inaug. Diss. Bonn, 1913.
9. Fabris, A. Aortite di Heller ed insufficienza delle valvole aortiche. *Pathologica*, 1909, i, 311.
10. Heller, A. Aorten Syphilis als Ursache von Aneurysmen. *München. med. Wchnschr.*, 1899, xlvi, 1669.
11. Jakob, A. Ueber die Endarteritis syphilitica der kleinen Hirnrindengefässe. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1920, liv, 303.
12. Karsner, H. T. Human Pathology. Philadelphia, 1926.
13. Koch, K. Eigenartiger Befund bei Aorteninsuffizienz infolge von Aortitis Syphilitica. *Virchows Arch. f. path. Anat.*, 1911, cciv, 470.
14. Larkin, J. H., and Levi, I. J. A pathological study of syphilitic aortitis and its serology. *J. Exper. Med.*, 1916, xxiii, 25.
15. Lupu, N. Untersuchungen über die mikroskopischen Veränderungen der Aortenklappen bei Aortitis Syphilitica. *Schweiz. med. Wchnschr.*, 1920, xli, 915.
16. MacCallum, W. G. A Text-Book of Pathology. Philadelphia, 1925.
17. Miloslavich, E. L. Contribution to pathological anatomy of syphilis. *Arch. Dermat. & Syph.*, 1925, xii, 41.
18. Waite, W. W. The nature and distribution of the lesions of syphilitic aortitis. *Am. J. M. Sc.*, 1927, clxxiii, 357.
19. Warthin, A. S. Primary tissue lesions in the heart produced by spirochete pallida. *Am. J. M. Sc.*, 1914, cxlvii, 667.
20. Warthin, A. S. Syphilis of the pulmonary artery. *Am. J. Syph.*, 1917, i, 693.

DESCRIPTION OF PLATE

PLATE 143

FIG. 1. Separation of the commissures and the involvement of the aorta in a segment of about 4 cm. in length.

FIG. 2. Extensive adhesions of the middle leaflet to the sinus of Valsalva. The midportion of the leaflet only is free.

1

2

Saphir and Scott Aortic Valve in Syphilitic Aortitis

PLATE 144

FIG. 3. Separation of the commissure and adhesion of the lateral portion of the leaflet to the sinus of Valsalva.

FIG. 4. Endarteritis obliterans of the vasa vasorum of the adventitia of one of the earliest cases.

FIG. 5. Mucoid degeneration of the inner portions of the media and intima taken from the same case as the previous section.

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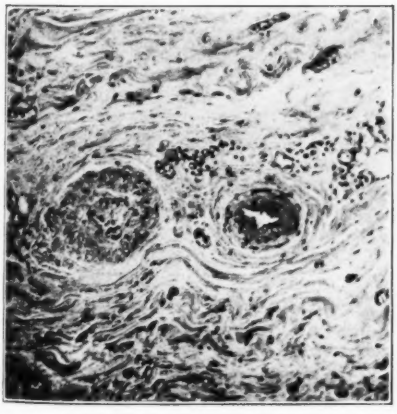

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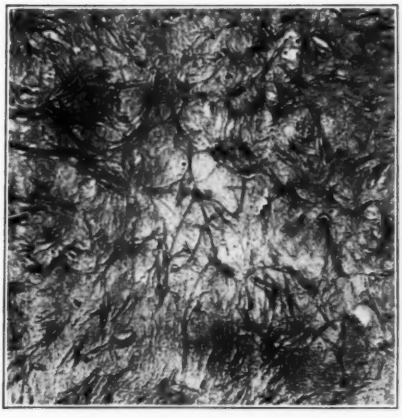

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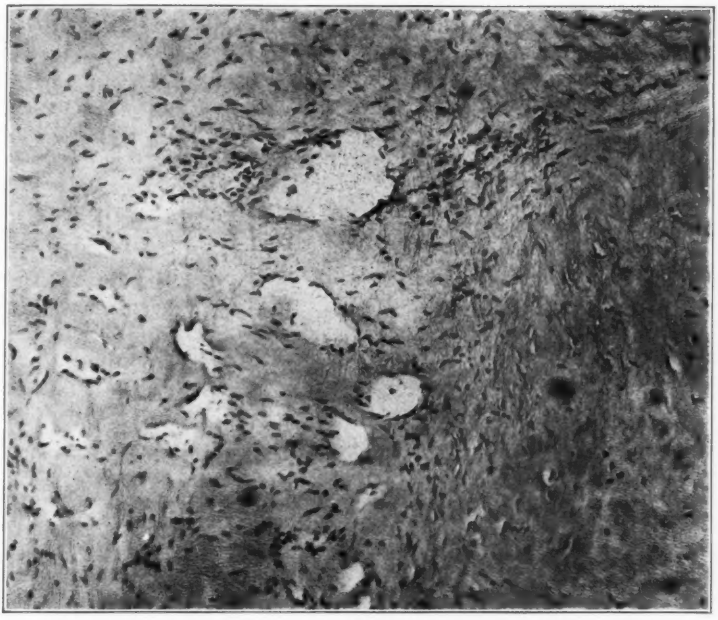
Saphir and Scott

Aortic Valve in Syphilitic Aortitis

PLATE 145

FIG. 6. A section taken from the commissures. Note the new formation of vessels.

FIG. 7. An area similar to the previous figure showing a much later stage. Note the vessels and the perivascular infiltration of lymphocytes.



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7

Saphir and Scott Aortic Valve in Syphilitic Aortitis

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SCIENTIFIC PROCEEDINGS OF THE TWENTY-SEVENTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ROCHESTER, NEW YORK
APRIL 15 AND 16, 1927

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

A Comparative Study of Basic Beef Heart Antigens in the Wassermann Test. Marjorie C. Albray (by invitation) and L. W. Famulener, New York City.

Abstract. Eleven antigens were prepared by various recognized methods from each of a number of fresh beef hearts. Comparative tests, to determine (*a*) relative hemolytic activities; (*b*) anticomplementary properties and (*c*) antigenic values, were carried out for each series.

The preliminary studies as reported were summarized as follows:

(*a*) *Hemolytic bodies.* The Noguchi acetone-insoluble antigen prepared by the *old method* and the Kahn antigen showed the least amount of hemolytic bodies, the Kolmer antigen, a slightly higher content, while the Noguchi acetone-insoluble antigen prepared by the *new method* showed a higher content, but not to a marked degree. The simple alcoholic extract antigen showed quite a high content of hemolyzing bodies. The addition or omission of cholesterol did not noticeably influence the degree of hemolysis in parallel tests.

(*b*) *Anticomplementary substances.* The Noguchi acetone-insoluble antigen, prepared by the *old method* averaged a slightly higher content of these bodies than the Kolmer or the Kahn products. The simple alcoholic extract antigen showed the least amount, but perhaps this factor was masked by the higher content of hemolytic substances present.

(*c*) *Antigenic values.* In each instance, the Noguchi acetone-insoluble antigen, prepared by the *old* or the *new* methods, showed the highest values (unit strength). Both occasionally showed a higher value when cholesterinized. The Kolmer antigen approached the Noguchi antigens rather closely in some instances, but the average values were less. The Kahn antigens were somewhat lower in value and in general paralleled the cholesterol content when this was varied, but not always (one exception). In general, the Noguchi and the simple alcoholic extract antigens did not show any decided increase in antigenic values on the addition of cholesterol (0.2 per cent) although exceptions occurred.

These studies are being continued with the hope of further elucidation of the question.

(*No discussion.*)

The Absorption of the Toxic Substance of Normal Goat Serum by Guinea Pig Tissue. J. D. Aronson, Philadelphia, Pa.

(*Abstract not received.*)

Local Hypersensitiveness. L. Dienes, Asheville, N. C.

(*Abstract not received.*)

Studies on the Absorption of Unaltered Protein. Matthew Walzer, New York City.

(*Abstract not received.*)

DERMAL TESTING AS AN AID TO DIAGNOSIS OF INTESTINAL PARASITIC INFESTATION. Matthew Brunner (by invitation), Albany, N. Y.
(*Abstract not received.*)

STUDIES OF ATTENUATION AND OF TOXIC PRODUCTION OF THE DIPHTHERIA BACILLUS. Mary W. Wheeler (by invitation), Albany, N. Y.

Abstract. These studies of attenuation of the diphtheria bacillus and its toxin production in synthetic media brought out certain interesting facts. One of six subcultures of the standard Park No. 8 strain of the diphtheria bacillus grew in Uschinsky's medium. This culture was avirulent and non-toxic and these properties could not be reëstablished. Agglutination and absorption reactions alone gave evidence that the strain was closely related to the original Park No. 8 strain. More than fifty subcultures of the standard strain and thirty other strains failed to grow in Uschinsky's medium.

Growth and toxin production of the standard strain was obtained in synthetic media containing the chlorides, sulphates and phosphates of sodium, calcium and magnesium with dextrose as the source of energy and peptone as the source of nitrogen. No growth occurred if purified serum albumin or fibrin, pseudoglobulin, primary or secondary albumoses, purified peptone, asparagin, histidine or glycocoll were substituted for the peptone.

In the medium containing only peptone and inorganic constituents, a reaction of pH 7.4 to 7.6 and the presence of 0.2 per cent dextrose was most favorable for growth and toxin production. It was interesting to note that a potent toxin was produced only when the medium contained calcium and phosphate ions heated together in the presence of peptone. In the preparation of a medium with a peptone with high calcium content, the addition of other calcium salts was unnecessary. If, however, such a peptone was rendered free from calcium, it was necessary to add other calcium salts. The calcium ions could be replaced by equivalent amounts of barium or strontium, but not by magnesium or manganese. The addition of colloidal calcium phosphate to a medium which was unfavorable for toxin production, stimulated toxin production, but the potency of toxins from which the calcium was precipitated was unaltered. No significant differences were observed in the nitrogen metabolism of toxic and non-toxic filtrates of cultures of the standard strain grown in a medium of exactly the same chemical composition.

Discussion

(Dr. F. M. Huntoon, Glenolden, Pa.) I wish to congratulate Miss Wheeler on her paper. The production of diphtheria toxin has caused much difference of opinion because every producer has had his own ideas and theories particularly about the question of leaving the muscle-sugar in or removing it. It was felt that the production of diphtheria toxin was an art and each worker felt that his method was the only authentic method. Now Miss Wheeler has shown that it may be produced in peptone water if conditions are controlled. This work has aided very considerably the well-being of the diphtheria toxin products.

(Dr. I. S. Falk, Chicago.) I would like to ask how long it takes for the toxin to appear and the earliest stage at which potent toxin can be obtained.

(Dr. B. Steinberg, Toledo.) We had some experience in trying to produce soluble toxins and found that the nature of the media, the pH and the presence of sodium salts made a great deal of difference. This paper explains a good many things to me.

(Dr. A. B. Wadsworth, Albany.) In addition to the fundamental observations on the conditions underlying toxin production, I think it is also interesting to point out, in this study of the attenuation of diphtheria bacilli, that the practically non-virulent strains could not be rehabilitated. Similar observations have been made on the pneumococcus with the attenuated strains that came from horses undergoing immunization. In the recent study of these strains, it was found that after a certain stage of attenuation the rehabilitation of the virulent type of culture is apparently extremely difficult, in fact, it was not accomplished in the experiments which were made.

(Dr. H. Zinsser, Boston.) I should like to ask Miss Wheeler whether in the attempt to rehabilitate the colony, her observations in any way corresponded to rough and smooth differences. This has just been discussed in the English Journal of Pathology. Has Miss Wheeler any observations which indicate that the non-virulent rough strains could be brought back to smooth? This is a condition which we have never succeeded in bringing about in our laboratory but which is quite commonly reported in the literature by others.

(Miss M. Wheeler, closing.) In reply to the question as to when the toxin appears: With the small quantities of medium used in most of these experiments, 15 cc., a potent toxin was present in from 24 to 48 hours.

At the time the experiments were made with the attenuated strain growing in Uschinsky's medium, no particular note was made as to whether this strain produced rough or smooth colonies. Colony formation, however, was not typical and I should say the colonies were rather hard and dry. Morphologically, the organisms were not typical but were very short and coccoid.

ACTIVE IMMUNIZATION AGAINST EXPERIMENTAL PERITONITIS. Bernhardt Steinberg and Harry Goldblatt, Cleveland, O.

Abstract. It has been shown in previous communications that following injection of colon bacilli suspended in physiologic salt solution, into the peritoneal cavity of dogs, these animals survived and at the same time showed a bacteremia. However, when colon bacilli were suspended in gum tragacanth and injected intraperitoneally the animals died but no bacteria were found in the circulation. It was postulated that death was due to absorption of toxic substances. The present communication is an indirect evidence of that assumption.

Dogs were immunized with small doses of living and killed colon bacilli. After varying intervals subsequent to the last immunizing dose, the dogs were given a lethal injection of gum tragacanth and colon bacilli. All the immunized animals survived.

Experimental peritonitis was produced by intraperitoneal injection of fecal material into dogs. A large number of dogs immunized with colon bacilli survived the injection of fecal material into the peritoneal cavity, while all the control non-immunized dogs died.

(*No discussion.*)

IMMUNIZATION OF HORSES TO ERYSIPELAS STREPTOCOCCUS TOXIN. John F. Anderson and (by invitation) George F. Leonard, New Brunswick, N. J.

Abstract. The results of the immunization of a group of horses with strains of erysipelas streptococci isolated by Dr. Konrad E. Birkhaug are reported. Using a combination of the injection of toxin from these strains of streptococci and the blood clot method of immunization, the horses have been bled for produc-

tion in an average period of three months. Their plasma contained more than 5000 neutralizing skin test doses of antitoxin per cubic centimeter. The use of this antitoxin in a series of cases of erysipelas was followed by a rapid decline in temperature, pulse and respiration, and disappearance and fading of the erysipelas lesions.

Discussion

(Dr. A. B. Wadsworth, Albany.) What was the virulence of the culture? You spoke of their being extremely virulent, how virulent?

(Dr. J. F. Anderson, closing.) I should have used the word toxic, extremely toxic, I judge by the toxin produced.

THE INCIDENCE OF VARIOUS SPECIES OF BACTERIA IN SPECIMENS OF SPINAL FLUID FROM CASES OF MENINGITIS. Ruth Gilbert and (by invitation) Marion B. Coleman, Albany, N. Y.

Abstract. In a bacteriologic study of 284 specimens of spinal fluid received since Jan. 1, 1920, from cases of meningitis occurring in New York State outside of New York City, only eighteen strains of meningococci were isolated. Three of these corresponded serologically to Gordon Type I, and three to Type II; one agglutinated equally well in Types II and III sera, and one in all three sera. The others were atypical strains.

The number of tuberculous spinal fluids was relatively very high, amounting to 167. Eighty-nine of the patients from whom these specimens were submitted were under 10 years of age.

Pfeiffer's bacilli were found in 23 cases, pneumococci in 33, and streptococci in 35; and in 8 instances organisms not commonly associated with meningitis were present.

It is important to note the relatively low incidence of meningococci in this series, which is even lower than that of *B. influenzae*. As the mortality in cases of meningitis due to *B. influenzae* is very high and there is some evidence that vaccine or serum treatment might be of value, a further study of such agents may be indicated.

Discussion

(Dr. H. Zinsser, Boston.) We have been studying immunization by the blood clot method during the last few years. The work that Dr. Lawson has done is very definite but not quite completed. I am rather surprised by the fact that the older studies of the influenza bacillus and the sub-groups do not seem to hold good in the studies we have made of the influenza bacillus which we have isolated during the last two years in Boston.

(Miss R. Gilbert, closing.) Those that we have isolated have not been definitely typed but we have most of them and the study can be continued.

SIPHONIC FERMENTATION TUBE FOR THE MORE RAPID ISOLATION OF THE COLON-TYPHOID GROUP OF ORGANISMS. F. B. Jones (by invitation), Montreal, Can.

Abstract. This siphonic tube was invented with the idea of reducing the time that is usually consumed in the growing and detection of these organisms. It consists of two glass bulbs which act as reservoirs and receive the fluid as it is displaced from the siphon by the collection of the gas produced during the proc-

ess of fermentation of the sugar. These bulbs are connected by an intervening siphon which consists of glass tubing of small caliber and is bent in the form of an inverted "V." As the gas is produced it readily collects in the acute angle of this inverted "V" and breaks off the direct communication, through the fluid, between the two glass bulbs.

This tube may be used as a fermentation tube generally wherever it may be found necessary to use such a tube.

(*No discussion.*)

THE FIRST ANNUAL REPORT OF THE REGISTRY OF TUMORS OF THE LYMPHATIC SYSTEM. G. R. Callender and J. F. Coupal, Washington, D. C.

Abstract. Forty-two tumors tentatively classified as shown in the following table have been received by the registry.

Tumor Registry, 1927

Tuberculosis			1
Hyperplasia in Lymph Nodes, Generalized			2
Lymphosarcoma	Plasma cell type	1	4
	With terminal leukemia	1	
	Typical	2	
Reticulum cell, malignant	Localized	1	6
	Diffuse	5	
"Hodgkin's Disease"	Typical	6	14
	Mixed cell	1	
	Sclerosing	5	
	Large cell	2	
Lymphatic Leukemia			7
Chloroma	Lymphatic Leukemia	1	2
	Myelogenous Leukemia	1	
Hyperplasia, Erythrogenetic tissue (Infant)			1
Carcinoma	Lung	3	5
	Testicle	1	
	Squamous cell	1	
	TOTAL		42

A classification of these tumors based on histogenesis is presented for discussion. A schematic outline of this classification is shown below. Pathologists were urged to send in samples of tumors of this kind for registration wherever it was possible for them to get the necessary data together, without reference to the person referring the case, it being understood that such cases were only available for study, and would not be reported without the consent of the pathologist registering them.

Discussion

(Dr. S. B. Wolbach, Boston.) I would like to ask Dr. Callender which vital stains he would like employed.

(Dr. Callender.) As I have done no work with the vital stains in this group of tumors I am not competent to suggest just which of the dyes should be used in the study. Janus green and brilliant cresyl blue have both been used, the former immediately on removal and the latter by injection before operation.

The clue perhaps to the method of study is contained in the rather numerous papers that have been written on the endothelial leucocyte or histiocyte.

(Dr. Wolbach.) Don't you think it would be advisable to advise a uniform technic? For your Council to state which vital stain or supra-vital stain would be best?

(Dr. Callender.) I do not think we are prepared to establish a uniform technic for vital staining but would refer that question to the Committee, Drs. Mallory and Ewing, for reply.

(Dr. James Ewing, New York.) Any good staining methods are all right.

(Dr. A. Plaut, New York.) Dr. Callender raised the question of erythroblastoma. Ribbert has published one case. Diffuse erythroblastosis seems to occur only in the newborn and I wonder if it belongs directly to the diseases which have been grouped here. Congenital erythroblastosis is an extreme exaggeration of the red blood picture present in the newborn. It is connected either with jaundice or severe congenital hydrops.

(Dr. Callender.) It was not my intention to call that a tumor, but just to point it out.

(Dr. Plaut.) In speaking about tumors, I only referred to Ribbert's case.

(Dr. Ewing.) I have been over all these cases and I am convinced that the registry is of value. I have learned a great deal from the forty or fifty cases. One thing I think highly important and that is, so far as possible, to have the bacteriologic studies. Several of these cases were very important bacteriologically. As for stains, every man has his own specialties in this field, and well fixed material stained by any method that is capable of analysis is the essential thing. Many of the specimens were not very well fixed. Bacteriologic studies and very thorough fixation of tissues seem the most important desiderata.

EXPERIMENTS OF TWO YEARS ON THE RELATION OF HODGKIN'S DISEASE AND THE ORGANISM ISOLATED BY GRUMBACH. Herbert Fox, Philadelphia, Pa.

Abstract. Some observations upon the relationship of diphtheroids to Hodgkin's disease and their pathogenicity, the occurrence of the lymphogranuloma in the lower mammals especially primates, and the blood picture of rhesus macacs are reported.

Repeated injections of the diphtheroid isolated by Grumbach from the blood stream of Hodgkin's disease during its active phase, into non-tuberculous rhesus macacs, during two years failed to produce in these animals any lesion that, grossly or minutely, resembled Hodgkin's disease. Injection of these cultures into guinea pigs confirmed in part Grumbach's work but it does not seem to the writer that the lesion in the lungs or lymphatic tissue should be called lymphogranuloma. With the monkeys the method was based upon the repeated introduction of large numbers of the bacteria so that constant assault upon the lymphatic tissue would exist. That the organisms were present in the body of the monkeys was indicated by the fact that No. 2 animal when sacrificed two months after the last dose, had the bacteria in the heart's blood.

Observations of the two animals during two years, and of the tissues, blood and serum of one of them at death fails to reveal any specific pathology indicating pathogenicity of the diphtheroid. The only and important specific reaction was a small amount of agglutinin. No complement fixation amboceptor appeared.

Hodgkin's disease has not been observed in the 3,000 mammals and 5,000

birds at this laboratory. Study of the lymphatic and myeloid tissues of primates indicates that the anatomic basis for the disease is as available as in man. Examination from an etiologic standpoint leads nowhere because it begins nowhere. The cases in domestic animals as reported in the literature are not convincing and are usually sarcoma or leukemia. The more acceptable cases might be myeloid hyperplasias dependent upon anemia.

It is not safe at present to attempt to state a normal blood count of the rhesus macac. There are variations due to factors not yet understood. It is best to determine the normal variations of each experimental macac and establish his own modes. Hemoglobin and red cells vary with the health of the beast and tend to rise after the animal comes from the dealers and becomes accustomed to new surroundings and gets good food. Repeated handling may affect the blood count; it certainly makes the temperature higher.

Leucocytes vary from 5,000 to 25,000 with an average of 10,000 to 13,000. Neutrophiles are the most numerous individual cell, having a percentage average of 44 to 54 per cent. Lymphocytes are next, their average being 33 to 47 per cent. Eosinophils and basophils are extremely variable and this work revealed no mode nor any reason for the variation. Many cells comparable to neutrophilic polynuclears have fine basophilic granulations. Mononuclears average 5 to 6 per cent. The leucocytes are not greatly affected by food. Sometimes the number drops, sometimes rises, after a meal following a fast.

The leucocytes of rhesus macacs perform a daily cycle of numbers with a high peak in the late evening hours. The course during the day hours is irregular but fails to show a distinct rhythm. The lowest counts seem to be from 11 A.M. to 3 P.M. The late evening rise is due chiefly to polynuclears. The leucocytes rise in numbers while the temperature is performing its normal night drop. This daily rhythm occurs without relation to food or sleep.

(*Discussed with next paper.*)

RELATION OF POLYNUCLEAR NEUTROPHILES AND THEIR REACTION TO X-RAY, TO CERTAIN LYMPHADENOPATHIES. Herbert Fox, Philadelphia, Pa.

Abstract. From the evidence here collected the leukemic group is composed of true chronic lymphatic leukemia, sublymphatic leukemia and leucosarcoma in that they have, with lymphocytosis, a low number of neutrophiles which are very susceptible to radiation treatment. Aleukemic lymphadenosis tends to have the percentages and numbers of neutrophiles and lymphocytes approach normal with relative insusceptibility to radiation. The cases have a tendency to caseous involvement, which is, however, not in our two cases attended by polynucleosis, and therefore suggest myelomas which are not characterized by a peculiar blood picture. This group of adenoses resembles somewhat in hematology lymphosarcomas. The tendency to final malignant stages as evidenced by the bony tumors in adenosis, increases the resemblance. The neutrophiles in lymphosarcoma do not fall consistently under radiation.

Hodgkin's disease is marked by a polynucleosis which is indifferent to radiation except in the malignant and febrile cases when these cells are quite variable. Our single cases of reticulum cell sarcoma and endothelioma had neutrophiles unaffected by radiation and of no definite numbers.

From these figures then, one can deduce that there is a leukemic group — true chronic leukemia, sublymphatic leukemia and leucosarcoma, and a sarcoma group — aleukemic lymphadenosis and lymphosarcoma, with distinct neutro-

phile picture and reaction to radiation. These are separate from Hodgkin's disease which varies with its type and from endothelioma and reticulum cell sarcoma. These features, if corroborated with a biopsy, will somewhat simplify the classification of a given case.

Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) I think it well worth while to emphasize the normal variations in the blood picture of any animal. Probably this is more noticeable in the monkey than in other laboratory animals. However, I feel that that should not prevent an attempt to draw deductions from hematologic studies if extra precautions are taken to standardize the counts in every way possible. If the same individual use the same pipette at the same time of day under conditions that are practically constant, many of these fluctuations will be minimized to a point where the results will be useful.

(Dr. B. Steinberg, Toledo.) A few weeks ago I had the opportunity to run through a series of twelve dogs. We had taken the temperature at two hour intervals, leucocyte counts, etc., and it was surprising to find how they varied. The temperature varied from 96° F. to 100° F. and the leucocytes from 4,000 to 12,000 in apparently normal dogs. We therefore could not find any normal standard to make out further experiments because of this variation in temperature and leucocyte counts.

(Dr. James Ewing, New York.) We have in our museum a uterus which was removed some years ago from a dog. The endometrium is about one-half an inch thick. I called this Hodgkin's disease but whether genuine or not I do not know. It has the histology and the gross anatomy of Hodgkin's disease.

(Dr. H. Zinsser, Boston.) I would like to say that in the case of rabbits a sort of fluctuation of the leucocyte count and the temperature appears but if one follows it consistently for long periods one can get departures from the normal. As far as diphtheroids of Hodgkin's or of any other disease, I do not think any bacteriologist who is familiar with diphtheroids pays any attention to an etiologic claim for a diphtheroid unless there is absolutely overwhelming proof.

(Dr. J. F. Coupal, Washington.) This paper brings out quite prominently the importance of quantitative studies of radiation in the Registry records as well as the value of the white counts. We do not need to annoy the clinicians too greatly about the history as long as we get the quantitative and qualitative record of the amount of X-ray and radium used.

(Dr. E. B. Krumbhaar, Philadelphia.) It would be interesting to know where significant changes had taken place and whether the polymorphonuclears in the young or old forms had been increased or decreased. I might take this opportunity to urge that a slight modification of the differential count will permit one to divide the polymorphonuclears into the old and young forms by a consideration of the nucleus and a very occasional metamyelocyte, so that the time for doing the differential count is only added to by five or ten minutes. In one case of low grade chronic lymphatic leukemia I made for several weeks an Arneth's formula and there appeared a constant reduction, the curve shifting rather to the right.

INTRA-VITAL STAINING AND PHAGOCYTOSIS IN HOGDKIN'S DISEASE, LEUKEMIA AND SARCOMA. Herbert Fox, Philadelphia, Pa.
(*Abstract not received.*)

ACUTE LEUKEMIA WITH EXTRA MEDULLARY MYELOID CENTERS. Harry T. Marshall and (by invitation) Katherine Woodward, University, Va.

Abstract. A negro child of five, after acute infections repeated over three months, died with features of acute leukemia and pronounced anemia. Pneumococcus on blood culture at necropsy.

The kidneys showed nodules, bright red in color like infarcts. Similar red splotches on dura mater. Malpighian hyperplasia in spleen. Pneumonia, organizing. No general glandular enlargement. Pansinusitis.

Microscopically, the red splotches in dura and kidney were not infarcts, hemorrhages or leukemic infiltrations. They were myeloid centers. Similar changes appeared in lung and to a less degree in peribronchial lymph nodes.

Conclusions are drawn as to the scope of the term "acute leukemia"; its applicability to this case, and its nature. The relation of findings to the definition of lymphocytes is indicated. Suggestions are made as to the bearing of this case upon current views of blood formation.

NOTE: Dr. Marshall defined tumor and leukemia before beginning his paper.

Discussion

(Dr. James Ewing, New York.) Did you find any traces of hemoglobin in these cells?

(Dr. Marshall.) Oh, yes.

(Dr. Ewing.) You didn't mention it.

(Dr. Marshall.) In the early picture the cells had distinct hemoglobin at all stages of hemoglobin formation from a deep basic cytoplasm, to the cells in the kidney interstices and lung interstices with various stages of hemoglobin.

(Dr. A. S. Warthin, Ann Arbor.) Did you examine the periosteum for similar cells?

(Dr. Marshall, closing.) No.

GENERALIZED RETICULAR-CELL SARCOMA OF LYMPH NODES, ASSOCIATED WITH LYMPHATIC LEUKEMIA. Maurice N. Richter, New York City.

Abstract. The purpose of this communication is to report a case in which a generalized tumor of unusual type, apparently primary in the lymphatic system, was associated with the clinical, hematologic, and histopathologic features of lymphatic leukemia.

The patient was a man, 46 years of age, with marked enlargement of the superficial lymph nodes, spleen and liver. The duration of the disease was said to be seven weeks.

Blood examination showed 98,400 white cells per c.mm., of which 90 per cent were lymphocytes, mostly of the small variety.

Death occurred three weeks after admission. The clinical diagnosis was lymphatic leukemia.

Necropsy: B. H., No. 11643. Summary of positive findings.

Generalized lymphadenopathy. The abdominal nodes formed a retroperitoneal mass which extended from diaphragm to pelvis, and from spleen to right kidney. Cervical, axillary, inguinal, epitrochlear, mesenteric and thoracic nodes were also enlarged. The nodes were discrete, soft and measured from 0.1 to 8.0 cm. in diameter. Some were white or gray, some hemorrhagic in

whole or in part. In a few were central areas of the same consistence as the rest of the node, but of canary yellow color. None was necrotic.

Spleen: 23 × 15 × 10 cm. Surface nodular. On section there were numerous white, gray or yellowish circumscribed nodules scattered through the splenic substance. These nodules appeared to be of the same type as the enlarged nodes. The largest nodule was 2.0 cm. in diameter.

Liver: Enlarged. Weight 2,660 gm. There were numerous white, circumscribed areas similar to those in the spleen, but smaller.

Bone marrow (rib): Hyperplastic, grayish red.

Microscopic Examination: The lesions in the lymph nodes, spleen and liver are of two types: (1) Diffuse lymphoid hyperplasia and infiltration. (2) Multiple nodular and diffuse neoplasia of reticulum cells.

The first lesion is identical with that usually found in lymphatic leukemia.

The second lesion is in the form of large mononuclear, occasionally multinuclear cells of varying size, shape and arrangement. In some sections the arrangement is that of a sarcoma composed of fairly uniform cells; in others, the presence of giant cells causes a superficial resemblance to Hodgkin's disease. However, there is no necrosis, fibrosis, or eosinophilic infiltration.

Both lesions are present in the liver, spleen and all groups of lymph nodes examined. The lesions of leukemia and of tumor may be seen side by side, often with intermingling of their cells. The cells of each, however, maintain morphologic independence.

That the tumor is composed of connective tissue cells is evident, but the cell of origin is difficult to determine. However, a study of the sections indicates that the cells probably arise from the reticular cells of the lymphoid tissues, and there these cells form the boundary of lymphatic sinuses, from reticulo-endothelium, although they do not actively produce reticulum fibers.

The bone marrow shows replacement of the marrow cells by diffuse lymphoid tissue.

Discussion. The association of leukemia with a tumor suggests several possibilities of etiologic relationship. This is particularly true of this case because one of the lesions (the tumor) apparently arises from reticular and reticuloendothelial cells, which are thought to be potentially capable of producing blood cells of various types.

However, the cells of the tumor and of the leukemia, although closely intermingled, remain morphologically distinct, without transitions from one cell type to the other.

That the lymphoid lesion is a true leukemia, and not a "leukemoid reaction" to the presence of a tumor, is indicated by the typical histologic lesions of leukemia in the organs. Likewise, there is no direct evidence pointing to the dependence of the tumor on the presence of leukemia.

The lesions in this case can best be explained by regarding them as separate conditions which, although closely associated, are morphologically distinct.

Discussion

(Dr. A. S. Warthin, Ann Arbor.) I believe that Dr. Richter's neoplasm belongs to the same group as those that we have seen in our material and have called leukemic sarcomatous Hodgkin's or leukemic reticuloblastomas. Of twenty-nine cases of sarcomatous Hodgkin's twelve developed a leukemic transformation. These neoplasms are all genetically related. They all belong to the

same group as far as their pathogenesis is concerned and represent different degrees of differentiation.

(Dr. J. F. Coupal, Washington.) We have taken refuge in the Registry of the museum by looking upon the blood, not as a tissue like some of the older histologists did, but as an organ which helps in understanding the cellular pictures found in this type of case.

(Dr. G. R. Callender, Washington.) I hope nobody who has a case approaching this in interest will avoid the Registry.

(Dr. A. M. Pappenheimer, New York.) I had the privilege of studying these sections. On looking up our material at the Presbyterian Hospital for similar cases, we found five which showed an histology identical with the tumor part of Dr. Richter's case. None of our cases, however, was associated with a leukemic condition of the blood. This might indicate with Dr. Richter that the two processes are distinct.

(Dr. George Baehr, New York.) I should like to lay emphasis upon the fact that chronic lymphatic leukemia is often a very chronic disease which exists throughout a large part of the patient's lifetime. In such circumstances it may occasionally happen that the individual will develop some other type of neoplastic disease. We have observed two cases of primary carcinoma of the lung in patients with chronic lymphatic leukemia.

(Dr. M. N. Richter, closing.) With regard to the diagnosis of Hodgkin's in this case I do not think the section is typical of the usual type of Hodgkin's that we see, although the eosinophils are not essential. In examining a good many nodes from all parts of the body, and nodules from the spleen, liver and other organs I might hope to find an eosinophil occasionally, but I was unable to do so in any tissue examined. Another thing of importance is definitions of the various conditions that we are talking about. As we must rely on the histology for the diagnosis, we ought to know what we mean by the clinical terms "tumor" and "leukemia." I fail to find very good definitions of these conditions, particularly of tumors and I do not know how to tell whether leukemia is or is not a tumor without knowing what a tumor is.

Diffuse Lymphoma of Mesentery Associated with Carcinoma of Colon.

Oscar T. Schultz, Chicago, Ill.

Abstract. In a man aged 54 years a mass was palpable in the left upper quadrant of the abdomen. The condition was believed to be an inoperable retroperitoneal sarcoma. Roentgen therapy was instituted, but had no detectable effect. Seventeen weeks after this admission symptoms of acute intestinal obstruction developed very suddenly. Colostomy was done. Death resulted from peritonitis three days later. At necropsy the entire mesentery was transformed into a layer of pale tissue 3 to 5 cm. thick, the liver and omentum were studded with carcinoma metastases, and an obstructing annular carcinoma was situated at the splenic flexure of the colon. The mass palpable during life was a portion of the thickened mesentery. The latter was free from carcinoma but had been transformed into a cellular tissue composed of closely placed lymphocytes. In places a follicular arrangement was present.

Discussion

(Dr. James Ewing, New York.) I would like to ask Dr. Warthin whether he thinks this is the same tumor condition.

(Dr. A. S. Warthin, Ann Arbor.) I do not think so.

(Dr. A. Plaut, New York.) I would like to ask how much fibrosis there was. One slide showed extensive fibrosis. Were there many spots like that?

(Dr. Schultz.) The section shown was from the periphery. There was not only no increase but a condensation of the bands of fibrous tissue near the surface.

(Dr. Plaut.) I asked for a special reason, because there was a condition of a tumor mass arising in the abdomen that was an "inflammatory tumor of the omentum," which Ewing has referred to as pseudosarcoma. A few years ago a case came into my hands and I found a large number of small foci of typical lymphoid tissue scattered throughout the extremely fibrosed omentum. There might be something similar in Dr. Schultz' case with the lymphoid tissue outgrowing the fibrous masses in the omentum. There must be some irritation but what kind of irritation seems to make little difference. This is only a suggestion.

(Dr. Schultz, closing.) In reply to Dr. Plaut I can only say that the striking thing was the diffuseness of the process; there were no nodules, but the entire mesentery and omentum were composed of a very cellular tissue of the structure of lymphadenoid tissue. There was no proliferative reaction whatever on the part of the stroma, and the lesion has none of the characters of a granuloma.

MALIGNANT LYMPH FOLLICLE HYPERPLASIA OF SPLEEN AND LYMPH NODES.

George Baehr and (by invitation) Nathan Rosenthal, New York City.

Abstract. A report of a study of six cases which represent a distinctive clinical and pathologic entity that can be differentiated from ordinary lymphosarcoma. The salient characteristics include: (1) lymphadenopathy due to hyperplasia of the germinal centers of the lymph follicles; (2) splenomegaly due chiefly to enormous enlargement of malpighian bodies, the weight of the spleen increasing up to 1,800 grams; (3) absence of abnormal cells in the blood; (4) absence of anemia or cachexia; (5) tendency to development of serous effusions in the pleural and peritoneal cavities due to the pressure of mediastinal or abdominal lymph nodes upon venous or lymph vessels; (6) absence of involvement of tonsils and lymphatic apparatus of the gastro-intestinal tract; (7) tendency to lymphatic infiltration in lacrymal gland resulting in unilateral exophthalmos. Aside from the absence of anemia and cachexia, the chief differential feature distinguishing the condition from lymphosarcoma is its origin multicentrically throughout the body in the lymph follicles, whereas lymphosarcoma arises monocentrically and spreads by lymphatic extension. Both lymph node and splenic enlargements respond with remarkable promptness to X-ray or radium therapy.

Discussion

(Dr. G. R. Callender, Washington.) We have two cases quite similar in the registry, one of which we followed for six years. Sometimes an overdose of X-ray gives rise to serious clinical symptoms.

(Dr. Baehr, closing.) I shall be glad to accept the correction as to terminology for there are undoubtedly more follicles in the spleen than are normally present,

but the malpighian bodies which normally are present are tremendously hyperplastic and although small collections of lymphocytes may have developed into additional follicles, I am at a loss to know what else one could call the condition except malignant lymph follicle hyperplasia. As to the caution about use of the X-ray, we have also records of a case which was treated with radium by someone else. The treatment was kept up long after the evidence of lymphatic involvement had disappeared, until eventually the patient developed a profound anemia and other evidences of radium poisoning.

THE NEOPLASTIC RELATIONSHIP OF HODGKIN'S DISEASE, ALEUKEMIC AND LEUKEMIC LYMPHOBLASTOMAS AND MYCOSIS FUNGOIDES. Alfred S. Warthin, Ann Arbor, Mich.

Abstract (not received).

Discussion

(Dr. H. Zinsser, Boston.) I wonder if Dr. Warthin knows how that sounds to a bacteriologist. It sounds like a catalogue of the ships in the second book of Homer. Perhaps Dr. Ewing may have some comments.

(Dr. James Ewing, New York.) I remember, Dr. Warthin, that paper which you presented in the early nineties. My criticism of the conclusions, I think, on the whole, was justified by the manner in which you yourself, just now, tore the entire classification to pieces. The only thing you left was the first term in the classification, "lymphoblastoma" and that I would demolish, as you do not seem to have the courage to do, by expressing the belief that there is no such thing as a parent cell of all these various neoplasms. I confess that Dr. Warthin's enormous material, which he has worked up for so many years with great care, may justify him in his rather firm and dogmatic opinions. However, I think Dr. Mallory could duplicate the numbers and possibly many of the varieties. Modesty prevents me from telling what we might show at Bellevue and New York and I think that Chicago could go a long way, so that on the whole one might encounter equally dogmatic opinions drawn from almost the same amount of material in many cities. This encourages me to retain some of my own notions about the subject which are not nearly as clear or as positive as Dr. Warthin's. The main ground for dissatisfaction with his Table is that in 1897 he proposed one name for this entire group of lymphoid reactions and he holds that opinion still. I think that is not going to help us much. I believe that as long as the etiologic factor or factors are missing, either bacterial, toxic or neoplastic, the only way of making real progress is to adhere closely to the morphologic distinctions in the hope that some day we shall find different specific agents associated with these pictures. It is my firm belief that that will be the outcome. I do not believe it is wise to assume that all leukemic processes are neoplastic or spontaneous non-altruistic growths. I believe many of them have distinct relations to infections. I have seen leukemic processes develop from pneumonia, acute tonsillitis and diphtheria and run a short and fatal course, or run a longer course into chronic leukemia. I think it entirely different from Hodgkin's disease which to my mind bears all the marks of a specific infectious disease. Although we may not be able to find clear differences in all cases, how are we going to make progress by throwing them all into one category? I would rather see the most minute differences emphasized and a classification based upon them until the time when the etiologic factors unify or subdivide the entire group.

(Dr. Zinsser.) I think it is almost my duty to ask Dr. Mallory whether he has anything to say.

(Dr. F. B. Mallory, Boston.) I shall be sufficiently destructive in the next paper so I shall refrain now.

(Dr. H. T. Marshall, University, Va.) In regard to one or two of these pictures that were shown which resemble the picture that I found in the infant, I was able to see indications of actual activity in the elongated cells between the renal epithelium, not within the tumor but in the new growth occurring between, which would not be tumor in the sense described by Dr. Warthin. Moreover, like Dr. Ewing I feel that Dr. Warthin has failed to take account perhaps of the adaptive changes that lymph nodes are capable of exhibiting. The only way to get any result would seem to me to be a tabulated series, studying the known irritants in measurable gradients. I think you will find that many of the changes are reactions in the nature of inflammatory adaptation.

(Dr. M. N. Richter, New York.) Now that we have definitions of the conditions which we are discussing I think perhaps we are in a position to talk of the etiology and the pathogenesis. I am particularly interested in the various blood pictures resembling leukemia. We know that various tumors metastasizing to bone marrow might throw out bone marrow cells into the circulation. I wonder whether the metastases to lymph nodes would not throw out various lymphocytes by a mechanical process without the necessity of assuming a separate neoplasm for their appearance in the blood. I would like to ask, in tumors associated with myeloid leukemia, what picture the bone marrow showed. Whether, for example, Hodgkin's in the bone marrow might possibly account for the irritation and the outpouring of myeloid cells.

(Dr. J. F. Coupal, Washington.) I think if we reiterate that these tumors arise from the various portions of the reticulo-endothelial system considered rather as an organ than as a tissue, we can justify all of Dr. Warthin's paper. If we remember that their stages of reversion proceed back to the primitive form, the embryoma, we can understand why these tumors exhibit so many cellular variations. The tendency for these tumors to form after chronic inflammation of this system, especially in its grosser lymphatics, indicates that it is subsequent to hyperplasia and hyperfunction rather than irritation and structural defects usually the cause of tumors in other organs. In addition, this system has repeated calls for a massive increase of physiologic action, as the rapid formation of large numbers of leucocytes and the vast increase in function shown in swelling of the lymphatics consequent upon infection. These marked variations of amplitude of function correspond to the marked, periodical, physiologic variations that occur in the breast and uterus, and which expose them to their high incidence of tumor.

(Dr. Zinsser.) I venture into this discussion reluctantly but I have been rather surprised by the frequent reference to the infectious origin which has been mentioned. This in particular applies to Dr. Ewing's reference in regard to Hodgkin's disease. I have been associated with some of the studies indirectly with Dr. W. C. Clark in which two chimpanzees observed by complement fixation reactions and bacteriologic studies were innoculated directly in the upper arm with Hodgkin's material under accurate control for many months. These experiments were completely negative. As a bacteriologist, I no longer have any confidence in the infectious origin of Hodgkin's disease.

(Dr. A. S. Warthin, closing.) I should like to lay out my old friend Ewing completely but I have great hopes for him. He will be something more than a

fundamentalist yet, I am sure. I will say that about the same time I was interested in the heredity of cancer he treated that idea even more roughly, but even now in his own book there are suggestions of an awakening belief concerning the heredity of neoplasms. We have exactly the same situation here, in the case of the lymphoblastomatous growths, Hodgkin's disease and mycosis fungoides, that we see in the case of other neoplasms. There is a great deal of evidence as to the intrinsic nature of these conditions, particularly with reference to the family history and the family incidence, three or four cases occurring in the same family in one or different generations, and I believe the problem here is essentially that of neoplasm in general, a genetic problem. The etiology is a problem of the germ cells and the qualities contained in them. As to infections of various kinds playing any part in the etiology of this group there may be some non-specific exogenous factor that will bring out the inheritable constitutional susceptibility but these processes do not in any way conform to infections. There is no inflammation in these neoplasms except occasionally a very slight secondary one. I think the spindle cells you saw in the slides are simply flattened cells. All of the patients are dead. The course of these affections is inevitably fatal, but the fatal issue may be prolonged by irradiation. These diseases spread by infiltration; they are all essentially destructive; they have no protective function; they have all the characteristics of neoplasms and none of an infectious process. They all represent neoplastic overgrowths of the parent tissues of the blood cell forming reticulo-endothelium or endothelium and differ only in degree of differentiation.

RETICULUM. F. B. Mallory and Frederic Parker, Jr., Boston, Mass.
(See page 517.)

MENSTRUATING UMBILICAL TUMORS. Carl V. Weller, Ann Arbor, Mich.

Abstract. Two umbilical tumors presenting all the characteristics of the so-called umbilical adenomyomas have been recognized in the Department of Pathology of the University of Michigan. One of these occurred in a woman 49 years old, while the other patient was 45. In the first case there was bleeding from a small nodular umbilical tumor at each menstrual period. In the second case, only histologic evidence of menstruation was available. These tumors showed the characteristic endometrial structure in respect to both glandular elements and surrounding cellular stroma but no smooth muscle belonging to the tumor was found in either instance. The absence of such muscle has been mentioned in other reported cases and the term "adenomyoma" is, therefore, a misnomer. Greater importance is attached to these tumors from the standpoint of the evidence which they afford as to the histogenesis of this group than to their clinical aspects. Of the latter, the most important is recognition of the fact that they are neither primary melanoblastomas nor metastases of malignant intra-abdominal neoplasms, conditions with which they are usually confused clinically. As to histogenesis, the occurrence of this tumor at the umbilicus cannot be satisfactorily explained as being due to endometrial implantation or to lymphagenous or hematogenous metastasis from proliferating endometrial growths in the pelvis. It throws doubt upon the theories of Sampson in regard to the origin of this entire group and seems to find its best explanation in a theory based upon a serosal origin.

Discussion

(Dr. A. Plaut, New York.) I would like to ask Dr. Weller and others as well, whether from this picture there was created an impression of a true neoplasm. I have the impression that these are not neoplasms. They are not circumscribed, not adenomyoma, but diffuse lesions. These glandular ducts which we have seen look really perfectly normal. They do not show signs of autonomous growth and the idea that such tissue should metastasize, I do not think, would occur to any of us in looking at slides of this character. As to the question of origin, I personally do not favor the theory of Sampson but rather the peritoneal theory which, I think, in some cases has been proven. But there is another possibility to be considered; these glandular ducts might be transformed lymph vessels, these epithelial cells might originate from endothelial cells. I fully realize what a shock this might cause to some tissue workers. I have seen a number of slides which led me to think of this possibility. Later I saw the excellent photomicrographs in the paper by Schiller in Vienna who comes to the same conclusion. If this theory is true, it can help us in understanding the diffuse multicentral character of adenomyotic lesions. If they are metastatic or embolic then we must assume that they migrate extensively in spite of their showing no signs of autonomous growth. On the other hand, the transformation of flat endothelial cells into higher columnar epithelial cells at several points in the same organ under the same stimulus seems to offer less difficulties of explanation.

(Dr. A. S. Warthin, Ann Arbor.) Have you ever found anything like this in the male?

(Dr. Plaut.) No, only in females where we can assume the activity of the female sex glands. Never before puberty and never more than a few years after menstruation ceased.

(Dr. Warthin.) Why then, only in one sex?

(Dr. Plaut.) I cannot answer that, but observations have led others and me to this conclusion. I have even found in older books drawings which present the same thing but without the same interpretation, of course.

(Dr. V. C. Jacobson, Albany.) I fully agree that the lesion is not a tumor. I should call it ectopic endometriosis. In other words, there are normal appearing endometrial glands plus normal stroma which at each menstrual period menstruate. I cannot conceive of strictly neoplastic tissue adhering to a normal function in such a normal way. As to why it is found in the region of the umbilicus I think the explanation in many of these cases lies in the fact that there are small umbilical hernia sacs present. Recently, I had the opportunity of studying a menstruating mass in the sac of an umbilical hernia. Endometrial tissue which had been set free in the uterine cavity had found its way into this sac. The endometrium fragments discharged at menstruation from the tubes or more often from ruptured endometrial cysts in the ovary wander around and find their way into certain places where they become anchored. The most important point in all this discussion lies in determining whether or not any of the endometrial cells cast loose at menstruation are alive. Novak and others feel that the tissue is all dead and if so Sampson's theory is wrong and Sampson's explanation just as dead. Dr. Sampson has shown (*Am. J. Path.*, March, 1927) that endometrial tissue can find its way into the uterine venous sinuses which are wide open during menstruation and a certain percentage of endometrial fragments become attached to endothelium and grow anew. They can in turn menstruate and give off more tissue which can be swept along even farther. I

think this article throws much doubt on Meyer's explanation of this condition. Another thing, if the peritoneal endometriosis is due to metaplasia of the peritoneal mesothelium why is it always or so frequently found on the under and lateral surfaces of the ovary and never on the anterior surface? The explanation probably lies in the fact that the fimbriated opening of the tube is on the under and lateral surface of the ovary. That is where the endometrial tissue is generally found. I should like to ask Dr. Weller if his patient had any menstruating ovarian cysts, or masses in either ovary. That is not necessary, however. In the case I have mentioned of menstruating endometrial tissue in the sac of an umbilical hernia, we searched the ovaries carefully and found no endometrial tissue. It was another instance of peritoneal implantation without any endometrial cysts in the ovaries which are usually responsible for this ectopic endometriosis. Many things are against the metaplasia theory. In the first place, it sounds very unreasonable to me to assume that such kaleidoscopic changes can take place in peritoneal mesothelium. The presence of menstruating cysts, the facts that the abdomen and particularly the pelvis contains menstrual blood and small masses of endometrium, and that these endometrial growths are usually found on the under and lateral surface of the ovary, point to peritoneal implantation of endometrial tissue. Also, I do not believe that the peritoneum forms decidua; the decidual cells are formed from the submesothelial fibroblasts.

(Dr. Weller, closing.) In the first place in regard to whether this growth is a true neoplasm or not: If this is not a neoplasm, a myoma is not a neoplasm. The fact that this neoplasm undergoes retrogression after sexual life is over is also true of a leiomyoma. In both of the cases described the neoplasm developed without trauma and there was no history of operation or of hernia. These neoplasms grow expansively. There is no limit to their growth until the age of sexual activity is over. So far as the origin and manner of growth is concerned these facts accord with many of the criteria for a true blastoma. The suggested possibility of implantation in an umbilical hernia does not help us very much. Why do such implants never get into the foramen of Winslow where there is a peritoneal pouch provided? There are many endometrial nooks and crannies which would correspond to an umbilical hernia. I do not believe that has anything to do with it. In regard to the continuity of these glandular structures, I am of the impression that they are independent structures. I have under way now a reconstruction which should settle that question. One of these neoplasms was removed nine years ago. As far as I can trace the history that patient did not subsequently develop ovarian cysts. My history of the case is not absolutely complete. In regard to the other case no internal pelvic examination was made.

SYNOVIOMATA: A HISTOLOGIC STUDY OF THREE TUMORS OF SYNOVIAL MEMBRANE ORIGIN. Lawrence W. Smith, Boston, Mass.

Abstract. Three tumors, occurring in relation to the knee-joint, and presenting too many points in common, both clinically and histologically, to be regarded as coincidental, are presented, as a type tumor to which no definite references can be found in the literature. Their histology, as based on their embryologic origin from mesothelium, is discussed. The type cell shows multipotential differential characteristics comparable to other mesothelial tumors, such as those arising from the pleura, pericardium and peritoneum.

Discussion

(Dr. A. S. Warthin, Ann Arbor.) Were these connected with any synovial cavity?

(Dr. S. B. Wolbach, Boston.) The third case was connected with the bursa beneath the patella. The other two cases developed in proximity to tendons.

(Dr. Jaffe.) Have you any idea of the frequency of these conditions?

(Dr. Wolbach.) These three cases and one other have come to our notice in about four years. One case Dr. Smith did not include in the series was sent to me from Vermont or New Hampshire. It received the same diagnosis and prognosis.

(Dr. V. C. Jacobson, Albany.) Have these any relation to the so-called xanthomatous tumors of tendon sheaths which contain many large cells filled with lipoid?

(Dr. Wolbach.) I do not know. Very probably a benign form. I become more and more convinced of what I frequently say to students that the so-called classification of tumors is simply matching tumors, about like what you do when your wife sends you down town to match a fabric.

(Dr. James Ewing, New York.) I believe these tumors represent a separate group, one of the common "rare" tumors. I found one recently which began well above the patella and I have seen one which was found in a long cyst in the middle of the thigh having the same general structure. I am sure it was of the same origin. I think there is evidence of a relation between these and the xanthomatous tumors which Dr. Jacobson mentions.

MALIGNANT MEDIASTINAL DERMOID WITH MENINGEAL METASTASIS. William F. Jacobs, Buffalo, N. Y.

Abstract. The number of mediastinal dermoids and teratoma reported in the last hundred years will approximate about one hundred cases. Of these, those definitely proved malignant will be about 10 per cent.

Carcinoma predominating and sarcoma next in order, two cases are reported of chorionepithelioma and two cases mixed, carcinoma and sarcoma.

The malignant change appears to occur not only in the solid teratoid growths, but also in the simple cystic dermoids.

Case report: A white adult male, 27 years of age; occupation — locomotive fireman. Complaint, — alleged fall, some fourteen weeks prior to decease when injury was received to left shoulder and chest, causing pain and shortness of breath; during final three weeks in hospital he developed signs of meningeal irritation, positive Kernigs with cervical rigidity. The spinal fluid showed increased globulin and a cell count of 34 with distinct web formation. The diagnosis at the time of death was pulmonary tuberculosis with secondary tuberculous meningitis.

Necropsy report: The body is that of a poorly nourished adult male, five feet eight inches, with weight estimated about 130 pounds, no signs of pulmonary arthropathy.

A tumor was found in the median line over the heart. It extended upward embracing the superior vena cava. The aorta was clear. The trachea and bifurcation formed the posterior part of the tumor. It measured 14 cm. in the vertical diameter, 8 cm. lateral and 8 cm. anteroposterior. On section it was found in part cystic and part solid. The cavity contained the usual sebaceous

material with hair. The inner wall presented a nodule from which the hairs were growing. The wall on section showed small areas of necrosis.

There was involvement of the peribronchial and paratracheal lymph nodes and the lower cervical group on the left side, also the lower pole of the left thyroid lobe.

The lungs, other than being markedly hyperemic, did not reveal any gross pathology.

The meningeal covering of the base and tips of the temporal lobes and its extensions into the Sylvian fissures were thickened, and presented numerous very fine granule-like nodules, suggesting tubercules.

The gastro-intestinal tract, liver, spleen, pancreas, kidneys, adrenals, prostate and testes were all negative.

Microscopic examination: The sections from different parts of the tumor mass revealed islands of hyaline cartilage, areas of striated muscle, smooth muscle, with a major portion made up of a dense hyalinized stroma, containing alveoli, more or less cystic with elongated columnar cells, suggesting respiratory mucous membrane. There are cystic areas lined by a very much modified stratified epithelium, showing both sudoriferous and sebaceous glands with hair follicles, and nerve fibers but no ganglian cells. The malignant phase of the tumor is represented by dense masses of adenomatous tissue in which the cells are small, cubical, at times showing considerable variability in size and chromatin content; the arrangement in the main exhibiting an attempt to form alveoli but often conforming to no order, showing no stroma, but being in solid masses, cords and strands; nowhere does there appear any limiting membrane; similar characteristics are found in the lymph nodes and thyroid.

In the lung sections, infiltration is found along the perivascular and peribronchial lymphatics. The infiltration extends directly through the vessel wall.

The involvement of the meninges was found to be in the form of strands or cords of cells resembling those of the primary tumor without special form or arrangement. They are generally found in close relation to the meningeal vessels with some few small round cells about them in the thickened meninges.

This case ordinarily would be classed with the simple cystic mediastinal dermoids. Histologically, however, we feel that it belongs to the teratoma.

Its malignant phase is represented by an adenocarcinoma, involving lymph nodes, thyroid, lungs and secondarily the meninges.

The factor of compensation, because of accident, prompted the postmortem examination.

The clinical diagnosis was pulmonary tuberculosis with secondary tuberculous meningitis.

The gross examination, while finding the primary tumor and immediate metastatic lesions, yielded a picture in the meninges that suggested tuberculous meningitis.

(*No discussion.*)

THE ENCAPSULATED TUMORS OF THE NERVOUS SYSTEM. (Meningeal fibroblastomas, perineurial fibroblastomas, and neurofibromas of von Recklinghausen.) Wilder Penfield (by invitation), New York City.

Abstract. The benign tumors of the nervous system arise from a specialized investment which separates nervous tissue from the rest of the body. They may be divided on histologic grounds into three groups: the meningeal fibroblastoma

(commonly called dural endothelioma), the perineurial fibroblastoma (solitary neurofibroma), and the multiple neurofibroma of von Recklinghausen's disease, although the two first named tumors are fibroblastic they are easily distinguished from each other microscopically because each retains the morphologic characteristics of the specialized connective tissue from which it arises. Only in the last group is nervous tissue to be found.

In addition to the standard methods for the study of these tumors much assistance has been gained by the use of the neuroglia and neurofibril stains of Del Rio-Hortega and of Cajal. The silver carbonate method for staining connective tissue fibers has been of particular assistance.

The type cell of the meningeal fibroblastoma is the fibroblast as pointed out by Mallory. Fibroglia fibers are formed within the tumor and when slowly growing, collagen is laid down by the neoplastic cells forming broad irregular fibers. Cell structure and arrangement resemble that of arachnoid granulations.

The perineurial fibroblastoma contains a different type of collagenous fibers. In these tumors the collagen is in the form of long slender wire-like fibers which appear in parallel. Similar slender fibers can be stained in normal nerve where they are laid down in the connective tissue. They have here frequently been confused with nerve fibers. Careful study of these tumors reveals no nervous or neuroglial elements in them. These tumors are most frequently found upon the eighth nerve and the nerve roots of the spinal cord. They are rare upon peripheral nerves.

In contradistinction to the above two tumor groups the neurofibromas are multiple tumors which appear in neurofibromatosis as an expression of a system disease which often involves a large number of nerves. Nerve fibers, derived from the nerve trunk, pass through the tumor and are surrounded by a tangle of reactionary connective tissue that is in reality a magnification of the widespread pathologic alteration of the nerves which takes place in this hereditary disease. Neuroglia cells are not found in these tumors, very rarely nerve cells may be present but they then resemble ectopic cells from a nerve root ganglion. Confusion has arisen from the fact that at times within these neurofibromas perineurial fibroblastomas may appear, possibly as a result of irritation of the perineurial connective tissue. These fibroblastomas may grow so large as to displace most of the neurofibroma tissue to the periphery. Sarcomas may likewise take their point of origin in these tumors.

In the gross also these three tumor groups have characteristic features. At operation the meningeal fibroblastoma is usually dark red, attached to the dura and often degenerated at the center. The perineurial fibroblastoma is attached to a nerve, is usually brown or yellow and degeneration, if present, takes place at a number of points resulting often in multiple small cysts with shining walls. The neurofibroma, likewise attached to a nerve, is more colorless, less vascular and on cross-section the degeneration which may take place is jelly-like and translucent. Fat granular cells are absent or infrequent in neurofibromas but common in degenerative areas of perineurial fibroblastomas.

Discussion

(Dr. James Ewing, New York.) Did Dr. Penfield state exactly what was the nature of those cells?

(Dr. S. B. Wolbach, Boston.) I would like to say that I cannot be quite satisfied with Dr. Penfield's interpretations. I have studied a great number of these

tumors which are being more carefully studied by Dr. Percival Bailey. We have a number of meningeal tumors of rapid growth. I cannot understand if these tumors are fibroblastic in origin why none of them resembles fibromas elsewhere in the body. There are tumors of the eighth nerve which approximate in appearance the tumors of the meninges. It is sometimes impossible to tell at first glance whether we are dealing with a meningioma or a neurinoma except by consulting the sheet that comes with Dr. Cushing's specimens. I am rather reluctant to accept the conclusion that these cells in both cases are of fibroblastic origin and prefer to believe in the ganglionic crest origin as some embryologists tell us.

(Dr. H. T. Karsner, Cleveland.) Dr. Penfield's discussion of the matter is interesting and clear, but I should like to ask him about the so-called neuroma or neurinoma. I have never been satisfied that such a tumor exists without the presence of ganglion cells, which, of course, are sometimes only revealed by examination of several blocks. Does Dr. Penfield propose to eliminate the ganglioneuroma with few cells and substitute for it the perineural fibroblastoma?

(Dr. Wolbach.) I have also in the last year had an opportunity to study two neurogangliomas. A recent one was very large, extending retroperitoneally from abdomen to chest and there is no question in regard to the presence of cells of nerve origin. Here again, in this encapsulated tumor there was the histology of perineural neurofibroma together with ganglion cells and I have been of the opinion that both types of cells come from a common precursor. I hesitate to quote Dr. Bailey but I believe he holds the same idea.

(Dr. Penfield, closing.) With regard to Dr. Ewing's question about the palisading cells: They are fibroblasts. I believe the fibers that one sees between and among these cells are collagen, reticulin if you like. Those same fibers can be stained on vessels where they usually wind about more. The same fibers can also be stained elsewhere in the collagenous ribbons of which they are a differentiation or precursor. I have been unable to stain any sheath of Schwann cells upon these fibers and they have not the form of nerve fibers in the central nervous system, which are more irregular in outline. They stain with connective tissue stains.

Dr. Wolbach spoke of recurring meningeal tumors. Of course, we know the meningeal fibroblastoma can recur. Also the fact that there are other types of tumors arising from the dura, which recur, seems an objection to the term meningioma which Dr. Cushing has proposed. Meningioma would include anything, I suppose, arising from the dura or which involves the dura but which would not of necessity belong to the histologic entity meningeal fibroblastoma. I cannot answer why these tumors are not like the fibromas seen elsewhere except that the cytologic picture resembles that of arachnoid granulations from which these tumors arise. Meningeal fibroblastomas tend to invade the bone and there is a tendency for arachnoid granulations to do the same thing, which I think Dr. Wolbach has pointed out.

The perineural tumors are sometimes difficult to interpret because of a great degree of degeneration. Fibroblasts which have undergone degeneration sometimes contain nucleoli and may give a superficial resemblance to nerve cells. The ganglioneuromas are certainly at times similar in appearance to the neurofibromas, not to the perineural fibroblastomas. Both contain nerve fibers and sheaths of Schwann cells. In regard to the term neurinoma that Dr. Karsner spoke of, the name signifies that the perineural fibroblastomas contain nerve fibers which is a matter of interpretation. If they are nerve fibers and contain

neoplastic ganglion cells then the name perineural fibroblastoma is a misnomer. I have tried to show that the solitary perineural tumor does not contain nerve fibers. The occasional tumor which contains ganglion cells belongs in the group of neurofibromas.

THE EFFECT OF HIGH VOLTAGE CATHODE RAYS ON LIVING TISSUE; AN EXPERIMENTAL STUDY. V. C. Jacobson and (by invitation) K. C. Waddell, Albany, N. Y.

Abstract. Forty white rats were exposed to the rays of the new Coolidge cathode tube, the area rayed being the unshaved abdominal skin. Voltages used were 100,000, 200,000, and 350,000 at 1 milliampere, the animals being one inch from the nickel window of the tube. The rats were killed at intervals of one minute to fourteen days following the exposure. At first the white hair in the rayed area is changed to yellow. This color gradually fades and the hair falls out. Desquamation and the formation of a superficial epidermal slough takes place, the necrosis gradually deepening until it reaches and sometimes penetrates into the muscle layer. The periphery of the burn shows practically no attempt at epithelial repair at the end of fourteen days. A dose of 200,000 volts for 60 seconds or 350,000 volts for 30 seconds, applied over an area one inch in diameter does not kill although the animal goes into general muscular spasm during the raying. The intensity of the histologic changes varies directly with the voltage. The first changes are degenerations in the epithelium of the skin and its appendages but very promptly the collagen of the corium and deeper layers is fused into a glassy mass which takes a deep black stain with hematoxylin, and orange-yellow with Mallory's aniline blue. Phagocytes attack and undermine the necrotic connective tissue thus exposing to the air the deeper tissues over which epithelium has great difficulty in growing. Elastic tissue is fused with the collagen bundles. An interesting point is that the cathode electrons or rays produce their greatest effect in a depth of only 1/10 mm. regardless of the voltage, dead matter (tissue) stopping the electrons perhaps even more efficiently than living tissue. The deeper tissue changes such as edema, vascular congestion and leucocytosis may be secondary X-ray effects or the reaction of the body to the presence of necrotic tissue. Direct raying of intestine destroys its muscle wall in 30 seconds and of liver its parenchyma in a depth of 1/10 mm. The profound change in the connective tissues and its effect on epithelial regeneration is being studied further.

Discussion

(Dr. A. M. Pappenheimer, New York.) What is the effect of the cathode rays on the bone marrow?

(Dr. Jacobson, closing.) I have confined the studies at present to the skin lesions, although in two rats direct raying of the exposed tibiae produced no demonstrable immediate effects. The rats, as a whole, seem to stand terrific voltage. They would go into convulsions while being rayed but come out of them without much apparent harm. A wide experimental field has been opened up by this tube. The cathode electrons produce remarkable chemical changes in substances, for instance changing castor oil into a solid, and the effect on living tissue indicates profound chemical alterations. The cathode particles themselves can apparently accomplish things not possible by the X-ray in the voltage customarily used. X-rays have never been used in voltages of 200,000.

THE BACTERIOLOGY OF THE BLOOD OF SWINE WITH PARTICULAR REFERENCE TO THE VIRUS OF HOG CHOLERA. Paul A. Lewis, Princeton, N. J.

(Abstract not received.)

THE DEMONSTRATION OF BACTERIOPHAGE IN OLD STOCK CULTURES. Gordon M. Kline (by invitation), Albany, N. Y.

Abstract. Previous investigations of old stock cultures have demonstrated bacteriophage in only a small percentage of cases, possibly due to the methods employed. When the technic used in examining stool filtrates for bacteriophage was applied to stock cultures, lytic substance was observed in 14 of 21 cultures studied. Typical bacteriophage, as identified by transmissibility in series and formation of plaques, was obtained from 11 of the 14 lytic cultures: namely, one *B. coli*, one *B. typhosus*, two *B. dysenteriae Shiga* and seven *B. dysenteriae* Mt. Desert strains. Tubes and plates, inoculated with the bacteria only, over a period of a few months varied from complete lysis to "normal" growth. In some instances, duplicate tubes and plates, inoculated at the same time from the same bacterial suspensions, gave these opposite extremes. This spontaneous appearance and disappearance of bacteriophagic action in these cultures associates the lytic phenomenon with other spontaneous microbic variations. The seven *B. dysenteriae* Mt. Desert strains gave both large plaques (3 to 3.5 mm. diameter) and small plaques (about 1 mm. diameter), indicating the presence of at least two distinct lytic mechanisms characterized as inherited processes rather than as chance contaminations with foreign parasites. One of the Mt. Desert strains was fished from isolated colonies ten times over a period of five years, but this procedure failed to eliminate either the lytic property of the culture as a whole or either one of the lytic mechanisms active in the strain before colony isolation. Although d'Herelle states that, "the bacteria of contaminated strains are but slightly or not at all agglutinable by a specific antiserum," the agglutinability of the cultures containing bacteriophage was normal with only one exception.

(No discussion.)

THE PARTICULATE NATURE OF BACTERIOPHAGE. J. Bronfenbrenner, New York City.

Abstract. Direct, as well as indirect microscopic examination of lysed cultures of bacteria, and especially the behavior of lytic filtrates in high dilutions and their ability to cause the appearance of discrete foci of lysis in bacterial cultures on solid media have led to the generally accepted view that the active principle of transmissible lysis (bacteriophage) is present in filtrates of lysed cultures in the form of discrete particles. The uniformity of the size of these particles, as found by different investigators and by means of different methods, has in itself appeared to many to be a strong indication in favor of the conception that the particles represent the units of an autonomous organized virus, as originally suggested by d'Herelle.

However, some experiments, particularly those showing that the active principle is capable of spreading from the focus radially, independently of the multiplication of bacteria, and independently of gravity, and that the rate of its spread is conditioned by the density of the medium, seem to militate against ready acceptance of such a view. Such radial spreading could be accounted for

by a working assumption that the observed particulate distribution of phage is only apparent and is due to its ready absorption on coarser colloidal particles of the medium. That such an assumption is more valid has been suggested by our experiments in which the number of particles endowed with specific activity of phage in a given volume of a filtrate was altered, depending on changes in the degree of dispersion of colloids in the medium. Experimental data presented in this paper strengthen this conception further by showing that the particles present in filtrates of lysed cultures of bacteria and endowed with properties of the phage are not uniform in size.

When filtrates of lysed cultures (bacteriophage) are subjected to prolonged dialysis under osmotic pressure against water, the presence of the lytic agent can be detected outside the membrane only during the first few days. The residue remaining inside the membrane contains the bulk of the original lytic agent, and yet it is no longer capable of diffusing into the outer solution.

The interruption of diffusion is shown not to be due to any alteration in the permeability of the membrane. Moreover, the residue fails to diffuse through a fresh membrane of similar permeability, while the dialyzed portion of the phage passes quantitatively through a new membrane. When ultrafiltration under pressure was substituted for dialysis, the residue on the filter could be washed repeatedly with water, or with buffer solution (pH = 7.4), without giving off into the filtrate any more active agent. However, if broth (pH = 7.4) was substituted for water, a renewed diffusion of the active agent resulted.

These results are interpreted as indicating that the colloidal particles present in the lytic filtrates, and apparently endowed with properties of bacteriophage do not represent autonomous units of the active agent, but merely serve as a vehicle on which the agent is adsorbed. They vary in size within limits wide enough to permit fractionation by means of ultrafiltration.

The fact that active principle can thus be shown to be distributed in the medium in a form of particles of different size does not necessarily deny its autonomous particulate nature, since the phage can conceivably be assumed to be a pleomorphic virus. However, the fact that the addition of broth to the nonfilterable residue (presumably composed of units of the agent of larger size) allows it to pass through the membrane which held it back before the addition of broth, is contrary to such an assumption, provided, as we have shown, the permeability of the membrane was not altered by the addition of broth. Such an effect of broth might explain the occasional findings of several workers who believe they have caused spontaneous production of phage by repeated filtration of bacterial cultures, heated lytic filtrates, and even sterile broth — substrata assumed by them to be free from bacteriophage before filtration.

(*No discussion.*)

THE STUDY OF INTIMATE MECHANISM OF THE LYSIS OF BACTERIA BY BACTERIOPHAGE. J. Bronfenbrenner and (by invitation) R. S. Muckenfuss and D. M. Hetler, New York City.

Abstract. When susceptible bacteria are introduced into a tube of broth containing moderate concentrations of bacteriophage, after a brief period of initial lag, the bacteria begin to multiply without showing any visible effect of the presence of the phage beyond possibly a somewhat accelerated rate of multiplication. With the rapid growth of bacteria the broth becomes increasingly turbid, until suddenly and rapidly, the turbidity disappears as the result of the appar-

ent disintegration of bacteria which can no longer be demonstrated either by staining or by cultural methods.

A number of investigators have tried to elicit the mechanism repsonsible for this sudden disappearance. As a result, there have been recorded in the literature no less than twenty hypotheses which can be roughly separated into three main groups.

The first group of hypotheses is built up about the original hypothesis of d'Herelle, who ascribed the sudden disappearance of bacteria to bursting, due to distention incident upon the intracellular multiplication of the *Bacteriophagum intestinale*. According to this conception, the bursting of bacteria accounted, at the same time, for the disappearance of bacteria as well as for the increase in the concentration of the phage in the solution. If this hypothesis is correct, there should be no lysis without multiplication of the bacteriophage, and *vice versa* — no multiplication of bacteriophage without lysis. The opposite, however, has repeatedly been shown to occur. Under proper conditions, it is possible to obtain lysis without an increase of phage, and an increase of phage without lysis. Moreover, many investigators have failed to observe this bursting, and some state that the swelling of bacteria observed by d'Herelle is not general enough to account for the disappearance of all the bacteria, that the bulk of bacteria disappears without undergoing any visible changes in morphology, and that swollen bacteria represent the cells more or less resistant to the bacteriophage, and are dissolved very slowly or not at all.

Another group of investigators ascribes the disappearance of bacteria to autolysis. However, they have failed to show the existence of a mechanism responsible for the sudden onset and extremely rapid progress of this autolysis. Besides, thus far all chemical analyses of the cultures, after the completion of lysis, have failed to indicate any increase in protein-split products, which would be expected if bacteria had become autolysed.

The third view postulates a special type of cleavage of the bacterial cell, which breaks up without chemical disintegration of its component parts. These cellular cleavage products are endowed with an independent power of multiplication and are capable of perpetuating a similar type of cleavage in other cells of the same species. Although this view, with various minor modifications, is held by several workers, it is evident that it is very hypothetical and not amenable to experimental inquiry.

Our own attempts to follow the progress of lysis by the direct observation of fresh preparations under the microscope, as well as in stained preparations, did not bring us to any definite conclusions as to the mechanism of lysis. However, we have been able to observe more or less definite increase in the size of bacteria under the influence of the phage. The use of special staining methods showed that the cell wall of the swollen bacteria remained intact in all cases, and in no instance were we able to detect any visible indication of its rupture or bursting. On the other hand, cytoplasm showed very marked changes during swelling. It took the stain less intensely and unevenly, so that in many instances it appeared to be segmented or beaded. When such cells were photographed unstained, by means of ultra violet light illumination, the cytoplasm appeared to be of uneven density. The swollen bacteria did not stain by Congo red (vital stain), thus indicating that they probably remained alive, even when extremely distended. The extent of swelling of individual bacteria, the relative proportion of swollen bacteria, as well as the actual relation between the swelling and the lysis were very difficult to establish, because the swelling and lysis of

bacteria go on simultaneously and continuously, and at different rates in the case of different individuals. The degree of swelling of individual bacteria varies to such an extent that the results of such an analysis must, of necessity, be highly subjective, which circumstance would explain the differences of opinion expressed by different workers on this point. The actual disappearance of individual bacteria from the field of observation in the fresh preparations is so sudden and rapid that it is impossible to state with any degree of accuracy which of the bacteria have disappeared.

It occurred to us that the extent of swelling and the relation of swelling to lysis might be more definitely established if instead of attempting to follow the changes of individual bacteria, a culture were considered as a whole. If an appreciable number of cells in a culture undergoes swelling, the relative volume occupied by the solids in this culture must increase, and thus affect the viscosity of the solution. Measurements were made both by means of a capillary viscometer of Ostwald and in the torsion viscometer of du Noüy. It was found that in general the viscosity of the mixture of bacteria with a corresponding bacteriophage increases steadily up to the time when visible lysis sets in, at which time the viscosity begins to diminish, until it gradually reaches the normal level. As calculated on the basis of changes in the viscosity of the solution, at the height of swelling, bacterial bodies may occupy a volume which may be from six to twelve times as great as the original volume occupied by them. If in place of living susceptible bacteria, one employs a culture of a homologous resistant variant, or heterologous bacteria, the viscosity of the solution remains unchanged. The heated bacteriophage which is devoid of its lytic power, does not induce swelling of bacteria and does not affect the viscosity of the mixture. The fact that after lysis the viscosity reaches its original level seems to indicate that swollen bacteria must be the ones which eventually undergo lysis.

The mechanism by which swollen bacteria disintegrate, however, remains to be established. Since in the study of fresh preparations one of the difficulties in following the lysis of individual bacteria is the fact that it is not possible to predict which one of the cells will disappear, and since one's attention is divided in observing a number of cells in a field, and moreover, since the actual disappearance of the bacteria is very sudden and rapid, it has not been possible to determine what actually takes place. It occurred to us, therefore, that if the lysis were recorded cinematographically, repeated projection of the film thus obtained would permit us to single out such cells as actually underwent lysis during the period of observation, and to follow them throughout this process. This method demonstrated that bacteria apparently swell to a varying degree, and then suddenly, within less than three seconds, the outlines of the cells disappear (burst ?) and in their place there remains an amorphous residue of low refractive power. This residue in turn disappears within a short time (about one minute), leaving only a few more or less refractile granules.

This rapid melting away of the bulk of the cytoplasm, which under ordinary circumstances is supposed to consist of semisolid colloidal, highly complex material, when considered in conjunction with the changes in the density and distribution of the cytoplasm, as indicated by pictures of fresh (ultra violet light), as well as of stained swollen bacteria, suggests that it became liquefied within the cell prior to the disappearance of the cell membrane. Since such a liquefaction could be accounted for only by some enzymatic process, we were led to investigate again the question of hydrolysis occurring during lysis. It seemed to us possible that the failure of earlier attempts to detect such hydrolysis might

conceivably have been due to the fact that the material subjected to analysis consisted of culture medium too rich in various products of hydrolysis to permit the detection of a possible small increase due to the lysis of bacteria. We therefore grew bacteria in the presence of the phage on synthetic medium devoid of all protein, and under this condition we have been able to detect unmistakable evidence of an increase in amino nitrogen as a result of the lysis of bacteria.

(*No discussion.*)

TULAREMIA, HISTOPATHOLOGY OF THE LESIONS IN MAN. Edward Francis (by invitation) and G. R. Callender, Washington, D. C.

Abstract. The lesions of tularemia in man may be divided into the local lesions at the primary point of inoculation, the secondary adenopathy subsequent to a primary lesion or without any evident point of entrance and the generalized lesions throughout the parenchymatous organs. The histopathology of these lesions was presented and illustrated. The early lesions are focal necroses, such reaction being followed relatively rapidly by endothelial proliferation and fibroblastic increase. The greater the duration of the disease the more marked is the fibroblastic response of the tissue to the infection. The similarity to certain types of focal lesions of tuberculosis was stressed and differential points considered. The histologic appearances alone are not considered diagnostic and should be differentiated from tuberculosis by special stains and inoculations and verified for tularemia by the agglutination reaction. Ten cases of the disease in man formed the basis of the study, three of which were fatal.

Discussion

(Dr. M. W. Lyon, Jr., South Bend.) I rise to a point in mammalogy. Dr. Callender says there are not as many rabbits in this part of the country as where he comes from. I am willing to concede there are not as many seen in public markets as one sees in Washington, but rabbits of the genus Sylvilagus are very common animals in the northern tier of the middle central states. The form occurring there is *S. floridanus mearnsi*, while the form commonly found around Washington and Baltimore is *S. floridanus mallurus*. This difference in races of rabbits may have some bearing on the distribution of tularemia, though the climatic factor is probably more important.

(Dr. H. Zinsser, Boston.) We have recently run into a case of abortus infection, an experimental infection in a young physician. In considering the diagnosis the question arose as to what extent agglutination overlapped in the two diseases. Can you give us a little information as to the difference in the serologic diagnosis and the similarity of the two infections in man?

(Dr. Callender, closing.) With reference to Dr. Lyon's remarks — I do not know that all rabbits are susceptible nor do I know of any work that has been done along this line. All rodents so far inoculated are extremely susceptible.

I am not sufficiently familiar with infection with *B. abortus* to discuss the similarities or dissimilarities between the two diseases.

With reference to the diagnosis, patients infected with *B. abortus*, *B. melitensis*, or *B. tularense* show agglutination of all three organisms, and the titer may not be significantly higher in the disease which affects the patient. Absorption tests differentiate easily and clearly so that there is no doubt in the mind of the bacteriologist.

ADDITIONAL EXPERIMENTAL EVIDENCE OF THE INTRACELLULAR DISTRIBUTION OF TUBERCLE BACILLI. Samuel R. Haythorn, Pittsburgh, Pa.

Abstract. Suspensions of virulent tubercle bacilli and Higgin's india ink were injected regionally in guinea pigs and rabbits and the transmission of tubercles, to other parts of the body by pigment phagocytes containing tubercle bacilli, followed. From pigmented subcutaneous tubercles in the groin, pigment cells bearing tubercle bacilli were traced to the regional lymph nodes, to the post-sternal lymphatic channels, to the anterior mediastinal lymph nodes, and to foreign body granulomas produced about inert substances in various subcutaneous areas. Pigmented tubercles produced interstitially in the ears of rabbits led to secondary pigmented tubercles of the submaxillary and peribronchial lymph nodes. The evidence obtained points to lymphatic distribution as being largely intracellular. The secondary tubercles are formed in part from wandering cells and in part from multiplication of local endothelium. Evidence is presented to show that a certain amount of intracellular distribution through blood stream is probable but is less important than in the lymphatics.

Discussion

(Dr. A. B. Wadsworth, Albany.) One question I would like to ask. I did not quite understand the technic — whether the tubercle bacilli were injected after or before the ink.

(Dr. Haythorn.) Mixed with the ink.

(Dr. Wadsworth.) I should like to ask Dr. Haythorn if he has any information as to whether or not the phagocytosis of the ink preceded or followed the phagocytosis of the tubercle bacilli in this experiment. I am much interested in the effect of the ingestion of the ink particles on the metabolic and other cellular activities and thought you might have some interesting observations in regard to the phagocytic activity of the cells that are full of ink.

(Dr. Haythorn.) The India ink cell is capable of taking tubercle bacilli.

(Dr. Wadsworth.) When well filled?

(Dr. Haythorn.) Yes.

(Dr. Wadsworth.) Was there absolutely no relationship between the phagocytosis of the tubercle bacillus and that of the ink?

(Dr. Haythorn.) I think a phagocyte is capable of taking up either and the presence of one does not interfere with the taking of the other. I know the presence of the pigment does not interfere with the taking up of the tubercle bacilli.

(Dr. H. Zinsser, Boston.) In connection with one point I have been interested — about the idea that the first contact of the tubercle bacillus was really with the phagocytes. Polymorphonuclears might carry it.

(Dr. Haythorn.) Dr. Gardner has done more work on that than I. As he is present, perhaps he will answer.

(Dr. L. U. Gardner, Saranac Lake, N. Y.) I have made a particular study of this problem in the peritoneal cavity of guinea pigs. Bacilli were found within eosinophils during the first half hour after inoculation. From one to four hours many were phagocytized by neutrophilic polynuclears and some by mononuclear leucocytes. By twenty-four hours practically all of the bacilli were found within mononuclear cells. I do not think phagocytosis by eosinophils is general as I have found it in no other animal except the guinea pig.

In highly allergic human beings acute inflammatory exudates are often seen which are composed largely of polynuclear leucocytes. Tubercle bacilli are sometimes very abundant within these cells. As examples may be mentioned certain phases of tuberculous meningitis, and acute tuberculous bronchopneumonia.

(Dr. Haythorn.) During the early stages the tubercle bacilli were only found in the large mononuclears but there is a stage in which the polymorphonuclears were very active during the caseation of the tubercle. I have not found any polymorphonuclear leucocytes carrying tubercle bacilli but I do not consider my experiments conclusive on that point.

(Dr. H. H. Permar, Pittsburgh.) The reason that Dr. Gardner did not hear the question at first was because we were discussing the reaction about the lycopodium spores. Was the reason the tubercles did not form around the lycopodium spores because the cells which had phagocytized tubercle bacilli did not move so rapidly as those which contained only carbon pigment?

(Dr. Haythorn, closing.) You put carbon pigment and tubercle bacilli into the groin and the large mononuclears take up a great deal of the pigment rapidly but there is still pigment which is not taken up immediately, and that is left free when the tubercle begins to form. The pigment cells included in the formation of tubercle are held together in the little granulomatous mass during development. There are usually some pigment cells in the surrounding tissue not included in the tubercle. When I waited for from fourteen to seventeen days and put in lycopodium I got tubercles formed by pigmented cells containing tubercle bacilli which I believe were freed by the breaking down of the nodules. At times I put in a little lycopodium. If I was too early I got only the pigment cells not at that time included in the formation of the tubercle, but after caseation I got tubercles formed by the pigment cells presumably freed by breaking down the tuberculous nodule.

PULMONARY BLASTOMYCOSIS; ITS SIMILARITY TO TUBERCULOSIS. REPORT OF TWO CASES. E. M. Medlar, Madison, Wis.

Abstract. The two cases upon which this report is based are cases of primary pulmonary blastomycosis. Skin lesions did not precede the pulmonary manifestations. Case I was a young male aged 17 years. He died of the infection about eleven months after the onset of the disease. He had generalized infection with many joints and bones involved. This case represents the acute type of blastomycotic infection.

Case II was a male age 65 who died from cardiac decompensation. He gave no history of pulmonary or skin lesions suggestive of either blastomycosis or tuberculosis. Necropsy showed pulmonary lesions indistinguishable from old tuberculous lesions.

On microscopic study no essential difference in the histopathologic lesions could be determined if the acute phase of the tuberculous lesion was compared with the acute blastomycotic lesion or if the chronic phases of the two diseases were compared. In other words, when the virulence of the infectious agent is considered the lesions produced in these two diseases are indistinguishable. Giant cell formation appears to be brought about in the same way. Caseation appears to be produced in the same manner.

From this study it appears that the reaction to the tubercle bacillus is not specific. A similar pathologic reaction occurs in blastomycosis.

Discussion

(Dr. L. W. Famulener, New York.) I would like to ask what culture medium and the age of the culture medium.

(Dr. Medlar.) I was not particularly interested in cultivating the organism but I found Sabouraud's medium best. I also found that factors such as moisture apparently govern the production of the aerial hyphae and fruiting bodies.

(Dr. Hans Zinsser, Boston.) You did not mean to say, Dr. Medlar, that you thought there was any danger of a pathologist confusing the two conditions.

(Dr. Medlar.) I do not see how you can make the diagnosis unless blastomycetes were present.

(Dr. Zinsser.) It seemed to me in our own studies that there was considerable difference — the diffuseness and the characteristics of the giant cells. I wondered if you thought that a competent pathologist might make a mistake.

(Dr. Medlar, closing.) In fact, most cases of blastomycosis resemble more an acute type of infection. Our first case was one in which the individual had a generalized blastomycosis which had lasted from a few months to a year. Our second case was one in which we had no idea how long the infection had existed. It shows a much more typical tuberculous picture histologically than the acute case. Without the presence of blastomycetes in this last case I could not tell the difference histologically between blastomycosis and tuberculosis.

COCCIDIOIDAL MENINGITIS: G. Y. Rusk and (by invitation) L. W. Buck, San Francisco, Cal.

Abstract. A report of four cases of basilar meningitis due to the above organism. History suggestive of pulmonary infection in two cases. In these, however, all pulmonary symptoms subsided and in one case which came to complete necropsy no infection was found outside of the central nervous system, except one possible collapsed organism in a section of lung. The cases ran a course of chronic hydrocephalus or other form of intercranial pressure, at times suggesting tuberculous meningitis, brain tumor, or hydrocephalus. Operative procedures were instituted in all cases. Demonstration of the material showing the characteristic organism.

(*No discussion.*)

SOME POINTS ON THE MECHANISM OF FILTRATION BY THE SPLEEN. W. L. Robinson, Toronto, Can.

Abstract. The histologic structure of the spleen, with its arterio-capillary system opening out into the pulp sinuses to bring the blood into direct contact with the vast network of reticulo-endothelial cells comprising the pulp, would seem to have as one of its purposes that of filtration.

Filtration experiments were done by intravenous injections and perfusions of fresh isolated spleens. As substances for filtration, India ink, acid and basic dyes, colloidal solutions of copper, platinum and silver, bacterial suspensions and red cells stained with eosin, were tried.

The capillary walls as they pass through the ellipsoids and continue on to open out into the pulp are permeable for fluids, colloidal solutions and suspensions of fine particulate matter. The red cells, as has been suggested before, apparently pass on through the end capillaries into the Ampulla of Thoma and the pulp sinuses. This serves as a mechanism for separating the plasma with its foreign material from the red cells.

Small quantities of India ink solution injected intravenously were practically all filtered out by the ellipsoids. Beyond the ellipsoids the first trace of India ink adherent to the pulp cells was found about the malpighian follicles.

Freshly isolated spleens perfused first with a 2 per cent solution of potassium cyanide filtered the India ink just as readily as the living spleen in intravenous injections. The process of filtration does not appear to be a vital one.

The pulp cells even when the pulp spaces were expanded to their capacity by the perfusing fluid, quite readily filtered the minute particles of India ink. The process of filtration is not, essentially, mechanical in nature.

In all cases the fine particles of foreign material were found firmly adherent to the filamentous processes of the pulp or ellipsoid cells and could not be dislodged by the blood plasma or the perfusing fluids. That the process might be one of adsorption is suggested by the fact that while India ink and platinum and silver colloidal solutions all carrying negative electrical charges were readily filtered out both in intravenous injections and perfusions of the isolated spleen, the copper colloidal solution which carries a positive charge apparently passed through the splenic pulp without filtration.

(*No discussion.*)

THE SPLEEN IN WEST AFRICAN YELLOW FEVER. Oskar Klotz and (by invitation) Winifred Simpson, Toronto, Can.

Abstract. Very little attention has in the past been given to the changes which occur in the spleen in yellow fever. The studies in the pathology of the disease have centered upon the lesions of degeneration which are particularly prominent in the liver and kidney, but which also affect the heart. One of the prominent characteristics in yellow fever is the lack of inflammatory reactions in any of the organs. The disease is one in which a toxemia severely injures and induces necroses which are commonly preceded by a fatty degeneration.

In a study of the spleen it was found that there was a fairly constant change to be observed in the fatal cases. The malpighian bodies show a marked disappearance of their lymphoid elements, while the endothelial cells within them appear more numerous. In the early stages of the reaction the germinal centers of the malpighian bodies may show some hyperplasia of their endothelial cells which is soon followed by a hyaline degeneration and necrosis. The lymphoid follicles tend to disappear and in their peripheral portions the endothelial cells in the sinusoids become free. These cells tend to enlarge and form irregular multinucleate masses. During the degeneration of these endothelial cells the chromatin of the nuclei becomes scattered through the cytoplasm and not infrequently resembles protozoan parasites. In the pulp spaces at some distance from the lymphoid follicles the tissues are injured but no definite hyperplasia of the cells of the reticulo-endothelial system can be made out. In those cases where the spleen has been severely affected, débris arising from the dead endothelial cells can be discerned in the sinusoids.

The reaction, although not a specific one for yellow fever, assists in making a differential diagnosis from other diseases which in the tropics may simulate it. It is distinctive from that which arises in relapsing fever and infectious jaundice.

Discussion

(Dr. J. Ewing, New York.) The splenic lesion which Dr. Klotz has so minutely described reminds me of the splenic lesions one sees in cases of intestinal intoxication. I am surprised too that one should discuss yellow fever without reference to leptospira.

(Dr. A. B. Wadsworth, Albany.) I would like to ask about hemorrhagic lesions.

(Dr. S. B. Wolbach, Boston.) I should like to know whether he succeeded in transmitting this form to guinea pigs.

(Dr. H. Zinsser, Boston.) Does anyone else wish to ask another question or the same question in another form? I will ask Dr. Klotz if he wishes to answer.

(Dr. Klotz.) The work which was carried out during the past year by the Yellow Fever Commission is not completed, and further studies are to be carried on during this and succeeding years. Hence the findings which have been reported to the present can only be considered in conjunction with those which are to follow. In answer to Dr. Wadsworth's question as to the presence of hemorrhagic lesions, it was noted that these were present in petechial or small blotchy areas in the skin, lungs, stomach, intestines and serosal surfaces, but their distribution and extent were very variable. We were unable to demonstrate leptospira in the tissues obtained at necropsy.

(Dr. Wolbach.) Was transmission to guinea pigs absolutely negative?

(Dr. Klotz, closing.) The laboratory work in bacteriology and immunology was carried out by Dr. Henry Muller who was in charge of the laboratories of the Commission, and Dr. Kligler. Guinea pigs were found not to be susceptible to infection by inoculation of blood from cases of West African Yellow Fever, and to the present no leptospira was isolated on culture media. The British officers both in Nigeria and the Gold Coast were most cordial in their coöperation, and gave every facility to carry on the work.

HISTOLOGICAL AND CHEMICAL OBSERVATIONS UPON THE ORIGIN OF THE POLYCYSTIC LIVER. Ernest Scott (by invitation), Columbus, O.

Abstract. From a review of previously reported cases and three cases observed in this laboratory, histologic investigation shows that polycystic liver is a condition associated with an increase in the periportal bile ducts. Previous investigators have attributed this increase to inflammation, neoplastic growth and congenital anomaly. From investigations of the three cases in this laboratory it is our opinion that the fundamental lesion is an inflammation in the nature of a periportal cirrhosis. Since all three of the cases reported were in adults, it is impossible to say if this statement holds for the embryonic type of polycystic liver. Physical chemical determinations on the cyst fluid reveals the absence of bile and proves beyond question that the cyst fluid is the product of selective secretory activity.

(*No discussion.*)

THE ETIOLOGIC STUDY OF THE PATHOLOGY OF EIGHTEEN CONGENITAL POLYCYSTIC KIDNEYS. James E. Davis, Detroit, Mich.

Abstract. The material studied was from subjects varying from fetal life to 65 years, one at five months, one at seven and one-half months, four at term, one

a day old, one adult 22 years of age, another 32, six between 40 and 50, three at 45, 55 and 65 years of age, respectively; two were from the negro race.

There were other multiple deformations in 50 per cent of the cases.

There was definite history of inheritance in 55 per cent of the cases. In two generations there were ten instances of congenital polycystic kidneys and three specimens were obtained from one family. Three of the cases were operated upon and only one has survived operation for a period of two and one-half years after her nephrectomy.

The material showed in all instances developmental defects in both cortex and medulla, the earliest formation occurring quite uniformly in the subcortical zones in kidneys, and in certain instances in liver and spleen. In the kidneys, delayed and defective development occurred in all parts of the majority of the nephrons. Delayed development of glomeruli, convoluted tubules and collecting tubules was evident in all cases, but in addition this deficient development was always found in areas of embryonic or mesenchymal stroma.

In all instances the details of differentiation phenomena were observed from columns of unassembled mononuclear cells in mesenchymal stroma to partial assembling of these cells as straight segments with here and there partial development of lumina to advanced cystic degeneration in such areas, whether found in the straight tubules, convoluted tubules or Bowman's capsules.

Conclusion. Structural etiologic defects preceding bilateral congenital polycystic kidney degeneration are due to delayed development of entire nephronic units and their surrounding stroma.

(*No discussion.*)

A Case of Hypoglycemia, Probably Produced by a Carcinoma of the Islands of Langerhans. H. E. Robertson, Rochester, Minn.

Abstract. This is a report of the pathologic conditions found in a case of hyperinsulinism and hypoglycemia which were studied and are to be published by R. M. Wilder, F. N. Allan and H. E. Robertson.

The patient, a physician aged 40, had had pains in the upper abdomen for a long period and more recently attacks of jaundice and weakness which could be relieved or prevented by the administration of sugar, the requirements increasing until just before death when not less than 25 gm. of glucose was necessary each hour. At postmortem examination a degenerated malignant tumor was found in the tail of the pancreas with metastasis to the adjacent lymph nodes and liver. The liver and kidneys were enlarged. Microscopically the tumor cells resembled those of the islands of Langerhans in structure and arrangement. The tumor tissue in the liver was found to contain insulin. The liver showed rich deposits of glycogen. The tumor was adjudged to be a functioning carcinoma arising from the islands of Langerhans.

Discussion

(Dr. James Ewing, New York.) It looks to me like parenchyma cells, but I do not understand why the glycogen was not burned as well.

(Dr. Robertson.) I believe that the glycogen was mobilized in the liver due to the huge amounts of sugar administered. Just how that happens I am not able to say. The facts are that the patient was suffering from hypoglycemia and hyperinsulinemia and it would appear that this tumor contains an insulin-like substance.

(Dr. Maude L. Menton, Pittsburgh.) Any special stains?

(Dr. Robertson.) They did not work.

(Dr. H. T. Karsner, Cleveland.) This report is of great significance and interest. Goldblatt (*J. Cancer Research*, 1921, vi, 277) reported a case of benign adenoma of the islet tissue but found few similar cases in the literature. The diagnosis, of necessity, rested upon a presumptive identification of the type cell. Robertson has the additional evidence of a special functional capacity of the tumor to aid in its identification. This case brings to mind the old question of preservation of function of cells after they have reverted to a highly active rate of multiplication.

RIGHT-SIDED AORTA (PERSISTENCE OF THE RIGHT AORTIC ARCH). Aaron Arkin, Chicago, Ill.

(*Abstract not received.*)

COARCTATION OF AORTA (ADULT TYPE) WITH COMPLETE OBLITERATION OF DESCENDING ARCH. Maude E. Abbott, Montreal, Can.

Abstract. A case is reported of complete obliteration of the descending arch at the point of insertion of ligamentum arteriosum with persistence of the fetal isthmus and anomalous (persistent left fifth?) arch. Bicuspid aortic valve, aortic insufficiency and subaortic stenosis in a lad of 14.

The collateral circulation was extensively developed and the ascending aorta had undergone aneurysmal dilatation with dissection and impending rupture of the right anterior wall. The diagnosis was made during life on the basis of the destructive symptomatology, which is discussed in the light of the necropsy findings. The literature on rupture of the aorta in coarctation of the adult type is briefly reviewed.

(*No discussion.*)

THREE SPECIMENS OF HEARTS SHOWING CONGENITAL LESIONS. B. L. Crawford and (by invitation) Edward Weiss, Philadelphia, Pa.

(*Abstract not received.*)

REPORT OF SEVENTY-THREE CASES OF PULMONARY EMBOLISM. J. S. McCartney (by invitation), Minneapolis, Minn.

Abstract. Seventy-three cases of pulmonary embolism were found among the 9,275 necropsies on record in the Department of Pathology of the University of Minnesota. They were divided into four groups (1, *a*) post-traumatic, 15 cases; (1, *b*) post-traumatic with operation in consequence of injury, 8 cases; (2) postoperative, 31 cases; (3) postpartum, 3 cases; (4) miscellaneous, 16 cases. There were 40 males and 33 females. The age varied from 19 to 83 years. When considered in relation to the number of necropsies done in each decade, this series shows practically the same incidence of embolism in all decades after the first. Age is probably not as important a factor as it is usually thought to be.

In the postoperative group, wound infection was present in 17 cases. In 27 of the 31 postoperative cases embolism occurred during the first two weeks.

Apparently the occurrence of thrombosis and pulmonary embolism after injury is not generally well known. One hundred and nineteen cases of post-traumatic thrombosis and embolism were collected from the literature. These

together with the twenty-three reported here make a total of 142. From these 142 cases certain general conclusions are drawn. (1) Post-traumatic thrombosis and embolism are not at all rare; (2) a simple fracture was present in a majority of instances, but only rarely a compound fracture; (3) in a number of instances there was only minor bruising of the soft tissues without fracture of a bone; (4) the thrombus always developed at the site of the injury; (5) the interval between trauma and pulmonary embolism is longer than that between operation and embolism; (6) the age distribution seems to be about the same as that of other forms of pulmonary embolism; (7) injuries resulting in embolism usually involve the lower extremities.

In postoperative thrombosis and embolism the primary site of the thrombosis may lie within the operative field or be distantly removed from it. The iliac, femoral and pelvic veins are the most common sites of the primary thrombosis. The veins of the left side are most often involved. Postoperative pulmonary embolism usually follows operations below the level of the diaphragm, and only rarely those above the diaphragm, and is most common following operations on the prostate, intestine and biliary tracts.

Thrombosis and embolism develop in a great variety of medical conditions, and the thromboses are usually situated in the veins of the lower extremities, as in postoperative cases, often independent of the major anatomic lesions.

Discussion

(Dr. M. W. Lyon, Jr., South Bend.) I would like to ask if figures for simple and compound fractures have been corrected for the frequency to the one and the comparative infrequence of the other.

(Dr. McCartney.) I took all the cases here in one group without making correction for the difference in incidence of the two types. I did so for the reason that in the literature it is frequently stated that embolism is more likely to follow a compound rather than a simple fracture.

(Dr. W. W. G. Maclachlan, Pittsburgh.) In the cases which we see in Pittsburgh in the coal miners, where compound fractures are very common and often multiple, it is very rare in these severe fractures to meet with an embolism at necropsy. The few that we have seen have all occurred from minor fractures or fractures not in good position, and operated on. In the very severe injuries, and the coal miners get terrific injuries, it is rare to find embolism.

(Dr. William Boyd, Winnipeg, Can.) Is there any explanation to offer about what exactly was happening in the case where the embolism did not occur for ninety days? Has anybody come across those postoperative cases where the patient dies with all the symptoms of pulmonary embolism but necropsy fails to reveal any embolus even if done with the greatest care. We have had two or three cases of that sort.

(Dr. H. L. Jaffe, New York.) I wonder whether any evidence of embolism has been noted in those cases in which there has been no injury but just manipulation of bone or joint in a closed operation. One sees suggestive cases in orthopedic hospitals following such procedures. The patient leaves the operating room and within a few hours after going to the ward dies with symptoms like embolism and careful necropsy fails to disclose the cause of death.

(Dr. McCartney, closing.) I have not any explanation to offer at all for the long interval between the injury and the embolism. It would seem that by the

end of three months you usually should have some organization of the thrombus in the vessels. I have made no microscopic examination of any of these specimens. This is simply a statistical study of the cases, no microscopic work being done. Certainly, in our service we see every now and again cases where the clinical history indicates pulmonary embolism but we do not always find embolism. I think that is the experience of everyone. I am sure I do not know how to account for it. None of these cases followed a manipulation such as Dr. Jaffe mentioned in orthopedic hospitals. I have seen such cases in the literature and probably the explanation is the same as in the others, namely that the vein is traumatized and the intima torn.

PULMONARY ARTERIOSCLEROSIS ASSOCIATED WITH PRIMARY CARCINOMA OF THE LUNG. William Boyd, Winnipeg, Can.

Abstract. The etiology of Ayerza's disease is still obscure. Some cases appear to be due to syphilis. In others the pulmonary arteriosclerosis is associated with conditions in which there is long-continued increase of pressure in the pulmonary circulation, such as mitral stenosis, emphysema and chronic pulmonary tuberculosis. In the case described in this paper none of these factors was present, but the pulmonary arteriosclerosis was associated with carcinomatosis of both lungs. The relationship between the two conditions is discussed.

Discussion

(Dr. A. S. Giordano, South Bend.) I wonder whether or not the microscopic sections of the prostate and of the rectum were carefully searched. The gross picture of the lung is a little bit unusual for a primary carcinoma of the lung. I was wondering if there was a possibility of primary carcinoma elsewhere. I have more than once been embarrassed to find what I considered primary malignancy in the lung, not to be primary in the lung at all.

(Dr. Alfred Plaut, New York.) Would Dr. Boyd like to give an opinion as what he thinks of the causal relationship or of the coincidence?

THE INVOLVEMENT OF THE AORTIC VALVE IN SYPHILITIC AORTITIS. Otto Saphir and (by invitation) R. W. Scott, Cleveland, O.

Abstract. The most constant histologic findings of the aorta in the region of the sinus of Valsalva are endarteritis obliterans of the vasa vasorum and perivascular infiltration of lymphocytes in the adventitia. These vessel changes lead to necrosis of the media and mucoid degeneration of the inner portion of the media and intima. The degenerated areas become organized as indicated by new formation of vessels and the presence of endothelial cells, fibroblasts and young connective tissue. The perivascular infiltration about the newly formed vessels and the presence of lymphocytes throughout show that the process does not undergo healing, but proceeds continually as a chronic inflammation. Older cases show hyalinization of these areas. The constant presence of the newly formed vessels prevents ulceration. The lateral portion of the aortic valve leaflets which according to Bayne-Jones is supplied by the vasa vasorum from the aorta shows similar degenerative changes as the intima and media of the aorta. Organization of both sides leads to adhesions between the lateral portion of the valve and the aortic wall of the sinus of Valsalva leading to the gross picture of a separation of the commissure.

(*Discussed with next paper.*)

THE HEART IN SYPHILITIC AORTITIS. B. J. Clawson, Minneapolis, Minn.

Abstract. One hundred and twenty-six hearts associated with syphilitic aortitis are studied to observe the anatomic changes in the valves, coronary arteries, myocardium and pericardium, and to note the immediate relation of these changes to the cause of death.

The 126 cases on the basis of their clinical courses and the pathologic findings at necropsy are classified as follows:

1. Aortic insufficiency, 48; 36.5 per cent.
2. Sudden death from closure of coronary orifices, 25; 19.9 per cent.
3. Rupture of aortic aneurism, 35; 27.7 per cent.
4. Gummata of myocardium, 3; 2.4 per cent.
5. Miscellaneous (death from other causes), 17; 13.5 per cent.

Syphilitic valvulitis (a cord-like thickening of the free margins of the cusps) and a separation of the aortic cusps at their attachments to the aorta are commonly associated with syphilitic aortitis. The gross injury to the valve regularly produces an insufficiency but never a stenosis. Narrowing of the coronary orifices to the extent of producing death is common.

Aside from the cases with rupture of an aortic aneurism and the few cases with gummata of the myocardium, death is practically always accounted for by the aortic valvular injury with insufficiency or the narrowing of the orifices of the coronary arteries. Sudden death with syphilitic aortitis is rarely due to a myocardial inflammatory condition.

Discussion

(Dr. Otto Saphir, Cleveland.) We have noted in our cases thickening of the midportion of leaflets of the aortic valve. We do not find there any changes or any signs characteristic of syphilis. We think that the cord-like thickening of the valves is just due to chronic inflammation caused by the regurgitation of blood after the insufficiency of the valve has been established.

(Dr. W. W. G. Maclachlan, Pittsburgh.) We have been interested in the relation of the aortic valves to aortitis, chiefly through some observations in cases of aneurysm where we found, clinically, no evidence of aortic insufficiency and in others at necropsy the presence of aneurysm with normal aortic cusps. We feel that there are a certain number of aortitis cases where the process does not go much below a point about one inch above the aortic ring. We agree with the findings of Dr. Scott and Dr. Clawson that lues produces a thickening of the aortic cusps, but some cases, even advanced to degrees like aneurysm, do not appear to show this change. After the age of 50 I think one must consider the change that may occur at that time of life due to sclerosis. We have been impressed on the clinical side by the fact that students invariably interpret an aortic regurgitation in an adult as being due to lues and to aortic valve disease, and do not seem to consider that an aortitis may be present with no valvular involvement.

(Dr. R. W. Scott, Cleveland.) We are very pleased to hear that Dr. Clawson's work, which agrees in every detail with our own observations of the last seven or eight years, indicates that whether a patient having syphilis of the aorta gets signs of heart disease or not depends not upon what happens in the myocardium but whether or not the process attacks the roots of the aorta and the valves. In regard to the distortion of the architecture of the leaflets one sees an occasional

clinical picture of aortic insufficiency although at necropsy the valve is little distorted. The ring itself is dilated perhaps but has perfectly normal appearing aortic leaflets. I wonder if Dr. Clawson has observed such cases, also whether or not he has seen a syphilitic distorted aortic leaflet in which the commissure was not widened.

(Dr. Saphir.) I wonder, too, if you have found any separation of the commissure of the two leaflets without hyaline plaques above the leaflets of the aorta.

(Dr. Clawson, closing.) From the microscopic condition of the valve I regard the pathogenesis of the thickening as inflammatory in character. The amount of proliferation which I showed you on the slide had not been produced from just some other injury. This inflammatory condition starts from the aorta and is known to extend beneath the endothelium of the valve on both sides of the valve but not from the sinus of Valsalva. There are many cases of aneurism without valvular involvement but as a rule the hearts are small and the valves in many of them are not involved and there is nothing to stimulate hypertrophy. In regard to the stretching of the orifice in three of my cases the cardiac failure cannot be accounted for on a vascular basis so we figured that in these cases stretching of the aorta must have had something to do with it. Unfortunately, the width of the aorta was not measured at the time of necropsy and I thought the measurement of the formalin specimen would not be correct. I have measured the aortic ring since in typical cases of aortic insufficiency with injury to the valve and have found that the diameter of the aortic orifice was normal. I have not worked up the microscopic structure of the aorta but in regard to the hyalinized plaques above the attachment of the valve you always have a proliferation which pushes the attachment of the aortic cusps apart somewhat. At first, it may not be hyalinized but may become so later.

PERIARTERITIS OBSOLETA NODOSA. A STUDY OF THE HISTOLOGICALLY HEALED END-STAGE. Aaron Arkin, Chicago, Ill.

(*Abstract not received.*)

A BLOODLESS METHOD FOR TAKING REPEATED BLOOD PRESSURE READINGS IN LABORATORY ANIMALS. Leone McGregor (by invitation), Minneapolis, Minn.

Abstract. Investigators attempting to produce chronic arterial hypertension experimentally find it necessary to take a long series of blood pressure readings on each animal. With the method of a cannula in an artery only a few readings are possible.

My method is a combination of the procedures used by Fahr and Allen. The rabbit is tied in the dorsal position. The phonendoscope is applied over the termination of the abdominal aorta, where it may be held in place by tapes. The cuff is wound around the lower abdomen just above the iliac crests, so that the phonendoscope is just inside the lower border. As Allen has pointed out, the sounds are more clearly heard when the phonendoscope is inside the cuff than when it is distal to the cuff as in clinical procedures.

The readings are taken in the usual manner. The cuff is inflated until the aortic pulse disappears. Then the mercury is allowed to fall. The first sound which comes through is taken as the systolic pressure. With a further fall in the

mercury the sound becomes softer. This is taken as the diastolic pressure. The aortic sounds are sharp and distinct and the pressure can be read as accurately as in man. The normal aortic systolic pressure in rabbits varies from 115 to 140 mm. Hg. Adrenalin shows the same sharp rise as occurs when the pressure is measured with a cannula in the carotid. The blood pressure can be read as accurately in monkeys as in rabbits.

(*No discussion.*)

A Method of Producing Experimental Chronic Hypertension in the Rabbit. A. H. Pederson (by invitation), Minneapolis, Minn.

Abstract. The kidney is approached by the posterior route. A longitudinal incision is made about one and one-half inches to the left of the mid-dorsal line, and the lumbodorsal fascia is exposed. Following the line of cleavage and separating the muscles in the direction of their fibers, the perirenal space is entered. With a little gentle pressure on the abdomen, the kidney is pushed out. The fatty capsule is stripped off and the renal vein and artery are identified and separated. An aluminum wire band is put around the renal vein, and sufficiently constricted so that the kidney becomes tense and purplish. To prevent the development of collateral circulation, the whole kidney is placed in a loose pouch of fixed animal membrane. The opening is surrounded by a purse string suture drawn about the renal pelvis and vessels. The kidney is then replaced, and the wound sutured in layers.

In the five rabbits that have survived the operation for two weeks or more, there has been a definite elevation of the aortic blood pressure, from 110 to 130 (normal) to 180 mm. Hg. or higher. The highest pressure recorded was 270 mm. Hg. The blood pressures were read by Dr. Leone McGregor by the method she has just demonstrated to you. One rabbit which has survived the operation for fifty days has a blood pressure of 180 mm. Hg. It is suggested that the hypertension is of reflex origin, due to increased resistance in the circulation through the obstructed kidney.

Discussion

(Dr. R. W. Scott, Cleveland.) I would like to ask what degree of constriction is produced by this band, and secondly, what is the nature of the material encapsulating the kidney.

(Dr. H. L. Jaffe, New York.) I would like to ask Dr. Bell if he constricted one or both veins and whether any changes in the blood followed the use of this method. Did any struggling of the animal have anything to do with the increase in blood pressure? My impression is that in rabbits blood pressure is much lower than those charts show, in the vicinity of 80 or 90.

(Dr. H. Zinsser, Boston.) I was interested in the normal blood pressure of rabbits because there has been a good deal of discussion as to arteriosclerosis present in the ordinary run of laboratory animals. There was a piece of work done not long ago about the changes in the artery due to repeated anaphylactic injury.

(Dr. E. T. Bell, Minneapolis.) Dr. Scott's question: The exact lumen of the vein was not measured but it is reduced to not more than one-sixth of its diameter and we watch it until you can just barely see the blood trickle through. The membrane is an ordinary thin paper condom. Only one vein was occluded. If you occlude both veins you get renal insufficiency and death from uremia.

Just one vein was used all the time. That produces no change in the blood chemistry. Of course you can take one kidney out and that has no effect. As to what the blood pressure is in the rabbit in the aorta: I heard a paper in the Physiology section yesterday in which blood pressures of 130 were reported in the aorta of the rat. In the rabbit by our method they were 110 to 140 as a general rule. This is a little higher than the pressure obtained with a cannula in the aorta. In discussing this with my physiological friends they thought it a little more accurate than the cannula inasmuch as we did not have to deal with the inertia of the column of mercury. Where the rabbit had a lot of arteriosclerosis in the aorta, and in many cases there was a great deal, there was no sclerosis in the small arterioles. With cholesterol feeding there is marked atheroma but that never goes beyond the large arteries.

ANALYSIS OF FOUR HUNDRED CASES OF PRIMARY HYPERTENSION. E. T. Bell, Minneapolis, Minn.

Abstract. Four hundred and twenty cases of primary hypertension that came to necropsy have been studied. Primary hypertension includes all cases with persistent or intermittent high blood pressure of unknown cause; also all cases with a heart weight of 500 gm. or more in males and 450 gm. or more in females, all known causes of cardiac hypertrophy having been excluded. In accordance with the manner of death the group may be subdivided into myocardial insufficiency, coronary disease, apoplexy, renal insufficiency and miscellaneous.

Ninety per cent of the patients were over 40 years old, and 74 per cent were over 50 years old at the time of death. In our necropsies 15 per cent of persons over 50 years of age had hypertension. The renal group are somewhat younger on the average than the rest. The proportion of males to females is about 1.4 to 1. The blood pressure tends to be highest in the group with renal insufficiency. Evidence is offered to show that all cases of idiopathic hypertrophy and dilatation of the heart with congestive heart failure are instances of primary hypertension. Gross sclerosis of the coronary arteries is more frequent and more severe in hypertensive than in non-hypertensive heart disease of corresponding age. There is some evidence that a large percentage of cases of clinical coronary disease are closely related to hypertension. It is highly probable that all cases of apoplexy on an arteriosclerotic basis belong in the hypertension group. The arteries in the parenchyma of the kidneys show some sclerosis in 97.6 per cent of cases of hypertension (severe in 61.7 per cent, slight to moderate in 35.8 per cent). The renal arterioles show sclerosis of varying degree in 88.1 per cent of all cases of hypertension. The coronary and miscellaneous groups show the lowest incidence of arteriolar sclerosis.

Discussion

(Dr. O. Saphir, Cleveland.) I would like to ask Dr. Bell if he found any fat in the arterioles of the kidneys.

(Dr. A. M. Pappenheimer, New York.) I would like to know what his ideas are on the relation of arteriosclerosis of the arterioles to arteriosclerosis in general. Is it a separate manifestation of the same thing or different altogether?

(Dr. H. T. Marshall, University, Va.) I would like to ask a question in regard to the fourteen cases in which there was no increase in the weight of the heart above the limit selected. The majority of our cases at least are accustomed to develop muscular size in proportion to the exercise but even some of the best

athletes have no muscular enlargement in spite of exercise. It may depend on family or individual peculiarity. I have often thought of that variety of pathologic condition where the adaptive hypertrophies are common. Sometimes one finds a condition of adaptive hypertrophy without increase in size. I would like to ask Dr. Bell if he thinks that increase in size of the heart would go with that number in order to prove that there was hypertension.

(Dr. Bell.) I cannot answer Dr. Pappenheimer's question as to the relation to senile arteriosclerosis. There are a great many observations to show that sclerosed peripheral arteries have no relation to high blood pressure. It is true that most diagnoses are made on clinical palpation only. We know that senile arteriosclerosis affects chiefly vessels in the extremities, the aorta and sometimes the large vessels of the circle of Willis and the larger branches of the aorta; and as far as we can judge in the cases which we studied there was no greater incidence of high blood pressure than in the normal. With disease of the small arterioles we get high blood pressure whether or not there is also senile arteriosclerosis. I do not have any definite opinion but I think the two conditions are on a different etiologic basis. I do not understand Dr. Marshall's question very accurately.

(Dr. Marshall.) I was referring not to increased heart but to the muscles of athletes. We frequently find athletes in training will show an enlargement of the muscles; after several years of training the visible muscles become markedly larger but the invisible muscles do not particularly tend to become large. This might account for the fourteen cases.

(Dr. Bell, closing.) Those cases are not proved but we must remember the blood pressures were not observed early. We cannot therefore conclude that they are cases of hypertension. There might be some other explanation.

THE ORIGIN OF THE ALVEOLAR PHAGOCYTE STUDIED IN PARAFFIN SECTIONS OF TISSUE STAINED SUPRAVITALLY WITH NEUTRAL RED. Leroy U. Gardner and (by invitation) David T. Smith, Saranac Lake, N. Y.

Abstract. A new method has been devised to preserve supravital staining with neutral red in paraffin sections. Animals are killed by air embolism and the dye is introduced either into the air spaces through the trachea or into the vascular system. This technic has been employed to study the histogenesis of the alveolar phagocyte.

Intravenous staining affects chiefly the cells in the framework of the lung; intratracheally it not only reacts upon these cells, but is sufficiently irritating to provoke a very rapid migration of alveolar phagocytes.

From the character of the cytoplasmic reactions to neutral red it has seemed possible to identify the alveolar phagocyte with the "septal cells" which project above the surface of the epithelium lining the air spaces. These septal cells are in turn classified as a form of clasmatocyte or connective tissue phagocyte.

From the study of this material we have concluded that the septal cells rapidly desquamate because of the presence of the irritating dye in the air spaces. The epithelial cells are not affected but remain in position. Within the alveoli the desquamated septal cells undergo morphologic and physiologic modifications resulting in the formation of typical alveolar phagocytes. New septal cells, replacing those which have migrated from the alveolar walls, are regenerated from connective tissue monocytes which in turn arise from dormant reticulum cells, stimulated by the presence of the irritating dye.

In support of these views the following observations seem pertinent:

Within five minutes after intratracheal staining the alveoli contain large numbers of phagocytes indicating a readily accessible source of supply. Such a source might conceivably be found in the following locations: (1) cells already in the alveoli; (2) cells in the circulating blood; (3) cells in the permanent structure of the lung. The first source, preëxisting alveolar cells, can be eliminated for two reasons; first, because phagocytes appear too rapidly for cell division to have taken place, and secondly, because the experiments were repeated on guinea pigs, only one day old, whose lungs not being previously irritated by inhaled dust do not contain free phagocytes. The second source, cells in the blood stream, can also be eliminated. The number of available cells remaining in the pulmonary vessels after the circulation has failed is too small to supply all of the free phagocytes which are observed. There remains, then, only some cell in the permanent structure of the lung, as a source of alveolar phagocytes.

The fixed tissue elements which have been suggested as possible origins are alveolar epithelium, capillary endothelium and connective tissue phagocytes or clasmatocytes. The reactions to neutral red of the first two types in no way resemble those of the alveolar phagocyte. Epithelial cells, studied in thick sections, exhibit only a few fine granules widely scattered throughout the cytoplasm; endothelial cells do not take up the dye at all, unless they are so damaged that the nucleus is stained. Furthermore, there is no evidence of any depletion of these cells after intra-alveolar irritation. Their number remains the same before and after intratracheal injection of neutral red.

The clasmatocytes of the connective tissue increase in number with the duration of irritation. They are apparently derived from extra vascular monocytes which are in turn produced by stimulation of dormant reticulum cells (Sabin). Clasmatocytes are found to some extent in the heavy areolar tissues of the trunks, and are very abundant in the alveolar septa. In the latter position they often project into the lumen of the air space between the epithelial cells. They are characterized by great numbers of neutral red granules, varying in size and color, scattered throughout their cytoplasm. After the dye has been injected into the trachea of the recently killed animal, the number of clasmatocytes in the alveolar septa is notably decreased. This is not so obvious in the living animal where regeneration is constantly taking place. Because of the similarity between the staining of free phagocytes and those in the connective tissues, and because of the depletion of the latter by the intratracheal injection of irritants, we favor this cell as the usual source of alveolar phagocytes.

However, it is not impossible that some of the immature connective tissue monocytes may migrate directly into the air spaces. This form, which is usually smaller than that in the blood, has been found in the alveoli, although it is rare. Its arrangement of neutral red granules in a rosette even more closely resembles that seen in the early free phagocytes than that in the cells of the septa.

Discussion

(Dr. Alfred Plaut, New York.) We have the term desquamation. All the books contain it. One speaks of desquamative pneumonia in tuberculosis and in other infections. I have never been able to find this conception compatible with the normal histology of the human lung. Our alveolar epithelium chiefly consists of extremely thin scales without nucleus and without distinct structure of the protoplasm. Smaller well structured pavement cells with nuclei are in

the minority. Therefore, it is difficult to trace the large numbers of nucleated cells which are found in the air cells of inflamed lungs to the alveolar epithelium.

(Dr. H. H. Permar, Pittsburgh.) Concerning the question of alveolar epithelium as the source of lung phagocytes, I too would be glad to hear nothing more. Some of us think we have settled that part of the problem, and I wish we might discontinue that part of the discussion. I believe one can see alveolar epithelium in certain pathologic states, tuberculosis, for instance, where there is a definite atelectatic process in the lung. We could discuss the origin of the dust cell all afternoon, but I am not yet willing to give up the endothelial origin. Dr. Gardner's stain does not stain the endothelium. I did not see, in the pictures, the capillaries in the alveolar walls. I should think you would see the nuclei of the cells lining the capillaries there.

(Dr. Gardner.) You can.

(Dr. Permar.) I did not see them in the pictures shown.

(Dr. Gardner, closing.) I might state that in the chronic organizing pneumonia so common in guinea pigs that one occasionally finds areas in which the epithelium has regenerated with the so-called fatty vacuolated type of cell. This type does not take neutral red at all. One can see connective tissue phagocytes lying behind the unstained epithelium.

DIFFUSE ADENOSIS OF VAGINA: A VERY RARE DISEASE. Alfred Plaut, New York City.

Abstract. At the age of 50 years, one year after menopause, a profuse vaginal discharge began. When the patient sought medical help two years later, a tumor-like mass protruded from the vaginal fornix. All the other pelvic organs were normal. Two small pieces were excised, and a diagnosis of adenoma of Bartholin's gland made. The tumor was considered as beginning malignancy. After several weeks the whole vagina together with the uterine cervix was amputated. The entire inside of the vagina was purple-red and showed a number of irregular eroded areas partly with central circular depression. The tissue in the eroded areas appeared granular; the underlying vaginal tissue and the paracolpium were of normal firmness and apparently not invaded by any foreign tissue. The posterior surface of the specimen was smooth.

The microscopic picture is not that of a tumor. Glandular ducts are scattered throughout the whole vagina in the connective tissue between the surface and the muscular coat. The surface epithelium is preserved at a few points only. It is normal squamous epithelium; no connection of it with glandular structures can be seen. The glands show much variation. Often large cavities are seen with smaller ones in the surroundings which obviously are parts of the larger one. Some narrow very long ones are found right under the surface. A low cylindrical epithelial cell prevails. The surrounding connective tissue is infiltrated; this accumulation of cells looks more inflammatory than like cytogenic tissue. Only a slight similarity with endometrial tissue can be noted. The tissue in the fornix is free of glandular structures. There is no connection with cervical glands and no connection with the peritoneal surface epithelium. A derivation from any embryonic remnants is highly improbable in such a diffuse lesion which in a homogenous way involves the whole vagina of an old woman. These glands look as if they had originated at the spot where one sees them now. Two such cases could be found in the literature, one with the diagnosis: diffuse adenoma of vagina, the other one called adenomatosis vaginae.

In our case the name adenosis was chosen because it does not prejudice anything and indicates that the condition is not of true neoplastic nature. No overgrowth of muscular tissue was found around the glandular ducts. Probably this disease belongs as a special form into the group of diseases the chief representative of which is the adenomyosis. Our patient was nulliparous.

Discussion

(Dr. A. S. Giordano, South Bend.) The microscopic picture resembled much the endometrial tissue in the so-called adenomyomas. Why could we not explain that on the basis of aberrant endometrium? We find aberrant thyroid in many places and why not aberrant endometrium? Certainly the glandular structure and stroma make it very suggestive of the relationship to endometrium. Going back to the history of this woman, was there curettage? The usual curettage means bleeding. I wonder if this was a type of menstruating endometrium present at that time.

(Dr. Plaut, closing.) I do not consider this tissue identical with endometrium. Endometriosis in the vagina has never been described. The glandular tissue in our case has no relation to endometrium proper. It cannot be a metastatic endometriosis. How can it be explained how it got there? If we should call it ectopic endometriosis we would have to go back to the embryonic state; it could not then be diffuse and restricted to the submucous layer.